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The Gift of

Sir Ronald Ross
Discoverer of the Transmission of Malaria by the Mosquito—Aug. 20, 1897

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RONALD ROSS AS MEDICAL DISCOVERER AND POET[1]

R. L. MÉGROZ[2]

The Little House, Ringmer Road, Seaford, Sussex, England

Through eyes and ears our minds are continuously beleaguered nowadays by propaganda, much of it serving narrow interests, even when it is honest. When we remember the truly great men to whom humanity owes its deepest debts it seems too often the result of an accident. In these crowded times, however, there are special reasons for remembering the late Sir Ronald Ross, and helping to celebrate what is in effect the Golden Jubilee of the greatest lifesaving discovery in the history of tropical medicine. I am not even sure that the qualifying word "tropical" is is necessary in this assertion, since Ross's peculiar and epoch-marking contribution was the discovery and demonstration of how malaria is carried.

As the title of this article suggests, Ross does not fit easily into familiar biographical classification. Originality, versatility and combative courage can be seen as outstanding characteristics in his career, but they were subordinated generally to a conscious purpose of serving humanity. Circumstances can influence a man's career but an innate genius determines the quality and effect of his work. Ross was born a poet and made into a medical researcher. His fame rightly rests on the results of his researches and not on his literary work, which nevertheless would make a great reputation for any writer. The reason is that after circumstances had impelled him into a doctor's job in India the poet in him joined forces with the humanitarian. He realized the appalling human suffering caused by disease, and especially by the "fevers" of unknown origin, and increasingly the energy of his genius was diverted from literature to medicine, or as he would have said, to the task of the Scavenger.

Ross was born in India because his father, General Campbell Claye Grant Ross, was there, in the Indian Army. More than a century earlier a member of the Ross family went to India and became a director of the East India Company. He and his brothers and sisters talked Hindustani with the Indian servants and English with their parents, whose intelligence did not correspond with the commonly accepted picture of the old Anglo-Indian type. Several members of the families of both the mother and father have shown unusual gifts in music and painting. The father, though a distinguished soldier in India, was a good water-colour painter, sang and also composed music to which the boy Ronald would set piano accompaniments. Ronald was sent home at eight years of age to continue his education, became a

[1] This article was written at the invitation of the editorial committee to commemorate the 50th anniversary of Ross's discovery of the method of transmission of malaria.

[2] Mr. Mégroz is author of the book "Ronald Ross, Discoverer and Creator" Macmillan Co., New York.

voracious reader of the English classics, especially the Elizabethans, and showed a passion for music. His school career however was undistinguished, and so was his subsequent study at St. Bartholomew's Hospital; for poetry, piano playing, and clay-modelling were serious distractions. He disappointed his father by not qualifying for the Indian Medical Service in time to reach India before his father's retirement from military service, and took a job as a transatlantic steamship surgeon—strenuous and badly paid though it was—on the strength of his only medical qualification, membership of the then Royal College of Surgeons. This was in 1880. The next year, by dint of studying during his cross-Atlantic trips, he passed the necessary examinations "without distinction" and received his commission in the Indian Medical Service.

In his *Memoirs* Ross recalled that his first post was at the Station Hospital, Madras, and the first things he did were to begin re-learning Hindustani and to find a good bookshop. He had ambitious schemes for private study, and his spare time for some years was devoted mainly to reading the European classics and philosophy, with a piano or a violin for recreation. Nevertheless it was not long before the mosquito pest thrust itself on his attention, as did also the contemporary ignorance and apathy. At Bangalore, where he was Garrison Surgeon for six months, until the spring of 1884, being much troubled by the attacks of mosquitoes he discovered that they were breeding in a tub outside the window, and got rid of nearly all of them by simply upsetting the tub.

"When I told the Adjutant of this miracle," he wrote, "and pointed out that the Mess House could be rid of mosquitoes in the same way (they were breeding in the garden tubs, the tins under the dining-table, and even the flower-vases), much to my surprise he was very scornful and refused to allow me to deal with them; for he said, it would be upsetting the order of nature, and, as mosquitoes were created for some purpose it was our duty to bear with them! I argued in vain that the same thesis would apply to bugs and fleas, and that according to him it was our duty to go about in a verminous condition. I did not know then that this type of fool is very common indeed. . . ."

During these early years of his service in India, although an ardent study of mathematics was added to his spare-time occupations, the problems of sanitation began to impress themselves on his mind, and a conviction was born in him, duly recorded in his poetry, that the work of the Scavenger must come before that of philosophers, artists and politicians, especially politicians. His journeys included visits to Burma and the Andaman Islands, and a second, prolonged, stay on the Andamans in 1886 caused him to start work on his first novel, the glowing romance, *The Child of Ocean*, which has the background of the islands. In 1887 he was again in Madras, suffering from the effects of prolonged overwork which he had imposed on himself, and the depression was accentuated by the delayed monsoon. He expressed his longing for escape to England in the series of brief poems entitled "In Exile" which became a sort of poetic journal of his experiences in India. He had not once applied for "privilege leave", and in 1888 he obtained a year's leave which he spent mainly in England.

At this time the work of Pasteur and Koch had established but recently the im-

portance of bacteriology. When he came home Ross was guided by his conviction gained in India of the need for efficient sanitary work. During his holiday therefore he studied for and obtained the new Diploma in Public Health started by the Royal College of Physicians and Surgeons combined, and then took a course of bacteriology under Professor Klein in London, early in 1889. This eventful holiday also included his engagement and marriage to Miss Rosa Bloxam, who returned with him to Bangalore. The young couple's luggage included a full box of type-cultures from Professor Klein's laboratory.

Before he obtained another year's leave, from 1894–95, he had a strenuous time, what with official duties and constant travels, the cares of a family, seeing through the press the first two novels he had written, and closer observation of mosquitoes, during which he made an important distinction between certain species, which afterwards proved valuable. In his verse he often expressed his concern as a doctor, as in "Indian Fevers":

> I pace and pace and think and think and take
> The fever'd hands, and note down all I see . . .

which concludes with a prophetic prayer:

> O God, reveal through all this thing obscure
> The unseen, small, but million-murdering cause.

In his paper on "Science and Poetry," read to the Royal Institution in 1920, Ross refers to the above verses and says that he did not then know of the years of toil which "seeking the laws" would mean, "and, still less, that success was to be obtained only by the most extraordinary good luck, without which we should, I think, have been still seeking in vain. Success was also due, I think, to the fact that the scientific work was first carefully designed, just as a work of art ought to be."

Because Ross had become an artist in verse and prose it would be quite wrong to think of him as a brilliant amateur of medical science, and, for instance, to compare him with the romantic Roger Naldrett of *Multitude and Solitude*, the novel by the British Poet Laureate, Mr. John Masefield, an old admirer and friend of Ross. Naldrett, a successful but high-minded author, ignorant of the rudiments of science, suddenly takes an interest in sleeping-sickness, goes on a hare-brained expedition with a companion into Central Africa, and makes a wonderful discovery of a serum within a year of his first lesson in the subject. Even "luck", which Ross regarded as an element in the success of his own researches, would not have sufficed if his scientific qualification had resembled the enthusiastic Mr. Naldrett's. What Ross meant by luck was the "trial and error" method imposed on him when he finally concentrated his energies and immense courage on discovering how malaria was transmitted. Success was to come because with a technique that was as good as it could be at that time he persisted in his labours in spite of illness and official discouragement until he may be said to have won chance to his side. When he came to London in 1894 with his family, on another year's leave, he had been thirteen years in the Indian Medical Service and had acquired some very definite views about the work that needed doing. He was the right man to benefit by the advice and encouragement he received before

going back to India from Dr. (afterwards, Sir) Patrick Manson, a leading figure in the new research into tropical diseases.

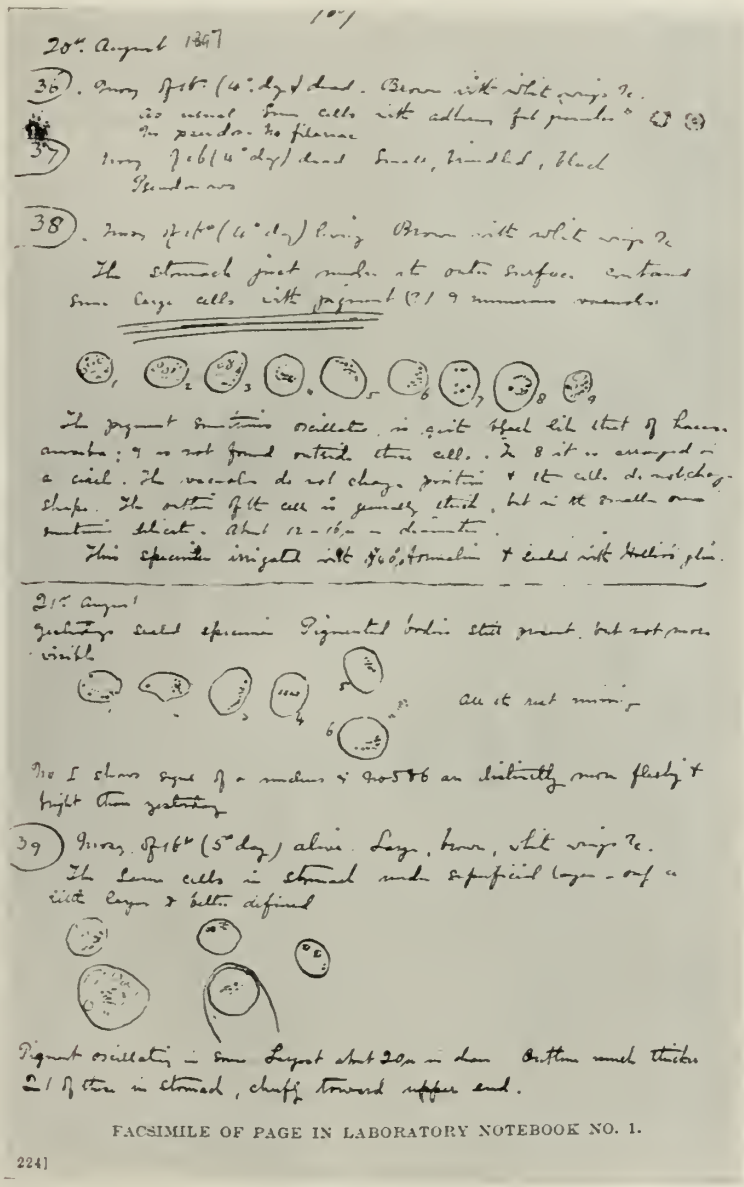
Fifteen years earlier the French army Surgeon Laveran had first observed the parasites of malaria in the blood of a human patient by studying blood specimens under the still-suspect and new-fangled microscope. This led to further observations by others, correcting some of Laveran's assumptions, and by 1885 the appearance of the parasites as dark spots of pigment inside pale round cells which develop and throw off spores had been noted in the blood of other animals and of birds. Experts in malariology today need historical perspective to realize the ignorance and apathy, not to speak of antagonism that surrounded researchers in this field during the last century. It is necessary to remember that only guesses had been made as to how the malaria parasite got into the blood and reproduced itself until the corpuscles were all attacked by the living spores. And though the guesses included mosquitoes, the old ideas were still favoured that "bad air" and stagnant water might be sources of the disease. Even Manson in his advice to Ross did not exclude these. If mosquitoes were looked upon with suspicion, little was known about them, and none knew that, for instance, the sort of mosquito which infected human beings might be different from those which infected birds or other animals. Indeed the possibility of avian malaria was treated with great scepticism.

Manson however did convince Ross that Laveran's parasites were what they were claimed to be. And Ross went back to India as on a crusade, vowed to prove or disprove the causal relation between Laveran's *Plasmodia* and a mosquito bite. It is worth recalling that during his 1894–95 holiday Ross had written a monograph which won the 1895 Parkes Memorial (triennial) Prize for the best essay on malaria. This essay showed convincingly the acute analytical sense, supported by an alert intuition, which enabled him to thread a path through mistaken and contradictory theories. He had determined to concentrate his efforts on the study of the parasite in the mosquito, rather than in the patient, a line of research so far completely neglected. Characteristic of the man, he had invented a small portable microscope which could be slung over the shoulders. A London instrument-maker brought it out for the inventor, and Manson's first letter to Ross says:

"Bakers (i.e., the instrument-makers) are going to lend me six of your microscopes for my lectures. They will be well advertised therefore, and I shall show my gratitude to you and Baker by giving them a puff!"

In the next two years Ross sought every opportunity of being sent to highly malarious places in India, and in his attempts to incriminate the right species of mosquito out of unknown hundreds, he more than once nearly died, more than once was tempted to yield to his depression at failure and official discouragement. He paid out a great number of his own rupees to Indians as bribes to allow him to take blood specimens, and captured and fed a variety of mosquitoes. He had, he estimated, dissected with care a thousand mosquitoes before the first light broke into his darkness. His wife and family were in England. To his wife as well as Manson he would write constantly of his experiments and labours. "During the four years since May 13, 1895", he wrote in *Memories of Sir Patrick Manson*, "I wrote 110 letters to Manson describing every detail of my work in about an average of one thousand

words a letter, and received back from him 55 letters (May, 17, 1895, to January 18, 1899), averaging about 650 words each, which I value highly . . ."



FACSIMILE OF PAGE IN LABORATORY NOTEBOOK NO. 1.

Ross records his discovery

Manson's theory that malaria might be spread by drinking-water on which a malarious mosquito had died, was not the only false trail which the ardent investigator pursued for a time. Ross also studied at length, at Manson's suggestion, the behaviour of the so-called free *flagella*—the whip-like tentacles of the male "crescent"

of the parasite which Laveran has observed. Neither Manson nor Ross realized that the "crescent" was a sexual form and that the *flagellum* thrown off was not an independent organism. Hence the searcher was for a long time puzzled by the elusiveness of the *flagella*, which seemed to disappear. He did not know that they entered the female crescents.

The time was approaching when he would be able to describe not only the behaviour of the parasite in human blood but its appearance in the mosquito. In this brief summary however to give a true picture of Ross's arduous progress towards truth it is well to recall some of his other work. He was sent in 1895 to the cholera-swept centre of Bangalore. His appearance there in charge of sanitary measures was comparable with the arrival of a cyclone. Within the first five weeks he had sent the Resident six emergency reports and extracted five thousand rupees from the Municipality for the necessary work. The epidemic ended and general sanitary investigation began, experience which proved valuable later when Ross had to elaborate malaria-control schemes. In January, 1896 the Resident received from Ross a report filling 126 printed foolscap pages which was still in use at the time of the first world war. Suggestive of the scope of this work is the *Memorandum on Open Sewage Drains* which he submitted to the slack Municipal Commission. Owing to the local conditions and the ignorance of the people the cholera returned and kept Ross's hands full. Just as the truth about malaria, when it was established, was to be neglected for many years while millions of people died of it, so now Ross saw and reported the neglect of Robert Koch's discovery sixteen years before of the cause of cholera. By 1896 certain workers in India besides Ross were beginning to apply the knowledge by disinfecting wells and introducing anti-cholera vaccine.

In the autumn of 1896 he was again working constantly on his malaria research and besides captured mosquitoes was using all kinds that he could breed from *larvae*. In the Spring of 1897 Surgeon-Major Ross was back in Secunderabad, from which he had been sent on the special mission to Bangalore. He had no higher post than his old one of regimental medical officer. Before returning to Secunderabad he had used two months' holiday to go at his own expense into the highly malarious region of the jungle at Ootacamund, and suffered a severe attack of malaria which he fought down with quinine and used as a source of useful data. He worked out a mathematical method of calculating the number of parasites in the blood and the time they took to breed, which subsequently proved accurate. It was only the beginning of the use of mathematics which he elaborated in his treatise, *The Prevention of Malaria*. At Ootacamund he caught scores of mosquitoes, but only one that he had distinguished long before as spotted-winged, which was an Anopheles. He had discovered five hitherto unknown mosquito parasites but not that of malaria, for he did not yet know which mosquito acted as host to malaria. Soon after his bout of malaria the investigator nearly died of cholera, and in the despondent mood of reaction after this illness he could not bring himself to look into his microscope for nearly a month. Instead, during another trying season of the delayed monsoon he added more stanzas to the "In Exile" suite of poems.

In this fateful year of 1897 Ross when he resumed work was the only researcher in the world investigating the mosquito theory. So precarious was the fight for

truth that would throw light not only on malaria but on other diseases caused by the mosquitoes! Besides a host of difficulties due to want of knowledge about the parasites and their complete life-cycle, the now familiar facts about the various kinds of Anopheles that do and do not carry malaria were unknown in 1897. Manson was making valiant efforts in London to bring influence to bear in the right quarters in India so that Ross's researches might be facilitated. They were not successful and one of the most important discoveries would have been terribly postponed if the searcher had not been animated by an ideal, served a power which he felt to be greater than himself. In July and August, 1897 Surgeon-Major Ross was again absorbed in his quest, organizing search parties for mosquitoes and their larvae, dissecting, recording, analyzing observations, speculating on the causes of each failure. Each mosquito that had been carefully fed on the blood of a malarious patient before being killed, required about two hours of concentrated work of dissection. He searched every micron of the tissues. "The things are there, and must be found! It is simply a matter of hard work," he wrote to Manson. Also a matter of spiritual energy. The great discoverers are not Peter Bells to whom a fact is a fact and nothing more. In the Latin meaning of the word, he went out as one goes to prayers and *considered* and was not ashamed to call the Stars "immortal Sons of God." He conversed with his soul, dramatizing the inner conflict, and when he looked at the stars his soul said:

> . . . For ever they go,
> Still onward, on and on;
> And that is why they know
> The victory's clarion.
>
> I said, "I am too weak
> To do more than I must."
> He said, "Then cease to seek
> And perish in the dust."

Working in his stuffy laboratory in Seconderabad on the 20th August, 1897, after several failures, about 1 p.m. he decided to sacrifice the last but one of his batch of mosquitoes, another Anopheles. The camp was quiet during the siesta. His eyes were strained, the miscroscope lens was cracked, sweat blinded him. He had set high hopes on the batch of dapple-winged mosquitoes which he had noticed stood with head sloping downwards, and he had found nothing. Should he go on? Yes. In his own words:

"I went carefully through the tissues with the same passion and care with which one would search some vast ruined cathedral for a little hidden treasure. Nothing! No, these mosquitoes also were going to be a failure. There was something wrong with the theory. But the stomach tissues remained to be examined—lying there empty and flaccid before me on the glass slide: a great white expanse of cells like a courtyard of flagstones—each one of which must be scrutinized. . . . But I was tired, what was the use? . . . But the Angel of Fate fortunately laid his hand upon my head . . ."

He resumed work, and almost immediately saw a dark stain that ought not to

have been there. He altered the focus of the microscope to make sure, and with rising excitement realized that he was looking at the pigmented malaria parasite in a mosquito. The next day he confirmed the observation. The parasite had grown larger. And in the last mosquito of the batch he found it again. After that he was soon able to show that the malaria parasite, sucked from a malarious person, passed through various stages, from the mosquito's stomach into the salivary gland and thence into the next human that the mosquito bit.

Immediately after that first sight of the promised land, Ross made some more neat entries in his note-book, sealed specimens to go with his report, and, characteristically, broke into verse: these three little stanzas, now part of his "In Exile" series, were copied in a long letter to Mrs. Ross telling her of the triumph.

This day relenting God
Hath placed within my hand
A wondrous thing; and God
Be praised. At His command

Seeking His secret deeds
With tears and toiling breath,
I find thy cunning seeds
O million-murdering Death

I know this little thing
A myriad men will save
O Death, where is thy sting?
Thy victory, O Grave?

It is by no means the best of the poems in the series but it is and deserves to be the most famous one. For it soon became apparent to the slowest intelligence that great human deserts of the globe might be made verily to blossom as the rose by applying the practical measures which Ross, quickly followed by others, was to devise for abolishing malaria epidemics.

An unfortunate interruption of his work by official duties again checked his progress though he lost no time in sending detailed reports to the authorities in India and at home. He was not in a position to complete the demonstration of his theory by infecting human beings with malaria, but in the spring of 1898 while at Calcutta he investigated avian malaria and was able to infect birds. His reports, now tracing the complete life-cycle of malaria parasites, appeared in the chief medical journals from July to September 1898, but official reaction was slow, and Ross meanwhile was sent off on another job.

The next few years were trying ones for the discoverer, who had to fight for recognition and also to get something tangible done in applying the new knowledge. He resigned from the Indian service, obtained a minor post at the new Liverpool School of Tropical Medicine, and set about making himself a nuisance to the authorities. His first big encouragment was the award of a Nobel Prize in 1902; it was only the second prize for medicine that had ever been awarded by the Institute. The lecture which Ross presented to the Institute on that occasion was translated into French

in 1928 by Dr. Charles Broquet, an authority on tropical diseases, who wrote in his Preface:

"Between these two dates, 1895–98, Surgeon-Major Ross completes *in vivo* in the mosquito the observations made *in vitro* by Laveran, of the emission of the motile filaments, and differentiates between the male and female forms of the *Plasmodium falciparum* in the insect's stomach; he notes the characteristics of the *Anopheles* and recognizes in this mosquito the presence of the *oöcysts* of the parasite of human malaria. Then, in a few months afterwards, in experimenting on birds, he demonstrates the transmission of the *Proteosoma* by the grey mosquito; discovers with the sporozoites (protospores) in the salivary glands of the *Culex* the last phase of the evolutionary cycle of the *Plasmodium praecox*, and proves that this *Plasmodium* is transmitted from the infected bird to the healthy bird by the *Culex*, and only by the *Culex*.

"Given the great resemblance of this *Plasmodium* of the bird to that of man, there was little doubt that the history of the two parasites was the same, the oöcysts in the fourth and fifth day of development, which Ross saw on the 20th and 21st day of August, 1897, in the two big Anopheles from Seconderabad, proved this similarity. It only remained to apply to man the results obtained with birds.

"As Manson wrote to him, Ross had hammered out the key; others might take the trouble to open the door . . ."

The French Doctor's tribute may be interrupted here to recall that two American researchers, MacCallum and Opie, had proved that the flagellated bodies observed first by Laveran, and then more carefully by Manson himself, were sexual forms, and that the throwing off of the flagella was not a process of dying as several investigators had asserted. This was an important link in the chain. But interest in the new knowledge spread rapidly early this century, and presently Ross's efforts to get mosquito-control measures adopted in densely populated malarious areas received powerful support. The most dramatic advance was made by the work of alert Americans who cleaned up Havana, and did that magnificent and self-sacrificing work which proved, on the lines of Ross's experiments, how yellow fever was carried by the *Stegomya*. There followed the successful mosquito-control measures at Panama conducted by William Crawford Gorgas on behalf of the United States Government. "It seems to me not extreme to say"—wrote Gorgas to Ross in 1914—"that it was your discovery of this fact (i.e. of how malaria is transmitted) that enabled us to build the Canal at the Isthmus of Panama."

In the first two decades of this century Ross himself visited many malarious districts as consultant, and developed his mosquito-control measures. One of the greatest achievements was in Malaya, where Ross's brilliant disciple, Sir. Malcolm Watson, supervised the extensive and complicated work which made the prosperity of the rubber plantations and transformed Singapore. But readers are probably familiar with Sir Malcom Watson's important book, *The Prevention of Malaria in the Federated Malay States*, an essential contribution to the history of malariology.

It would take up far too much space to detail the activities of Sir Ronald Ross until his death in 1932, but it is relevant to observe that his main contention of the neces-

sity for mosquito-control has again begun to make fresh headway against the medical school which has tended to find the solution in prophylactics. Quinine, of which there was never enough for efficient prophylaxis, has been almost superseded by a succession of new drugs whose usefulness stopped short of becoming a sufficient substitute for mosquito-control. It is true of the insecticide D.D.T. that at present it has the fault of not being sufficiently selective and when used has sometimes done much damage by killing bees and other useful life, but where as in the jungle such risks are much smaller, it may encourage those who regard anti-mosquito campaigns as too costly and troublesome. Moreover the best of the drugs, like the British paludrine, and the American chloroquine, must be of the greatest help towards the much to be desired conquest of malaria in all densely populated areas of the world. The reward in the shape of increased agricultural and other productiveness is so important that every malariologist must feel bound to support the efforts of the World Health Organization in the fight against malaria, and to see that the sanitary task of mosquito-control in all appropriate areas is given the highest priority. Ronald Ross, who died disappointed at our failure to carry out the necessary work that was made possible through his discovery would rejoice at last if enlightened self-interest should prevail. How long it will be before the world is freed from the scourge of malaria, he once said to me, "depends on how stupid people are."

Portrait Placque of Sir Ronald Ross on the London School of Tropical Medicine Building

BIOGRAPHICAL DATA AND BRIEF BIBLIOGRAPHY OF SIR RONALD ROSS; K.C.B., K.C.M.G., F.R.S., etc.

I. Born at Almora, Kumaon Hills, Himalayas, on May 13, 1857. Son of Col. (afterwards, General) Sir Campbell Claye Grant Ross, K.C.B., Bengal Staff Corps, and of Rosa (Bloxam) Ross

II. Died after serious illness and partial paralysis at Putney, London, on September 16, 1932. Cremated at Golders Green. Tomb in Putney Vale Cemetery, London.

III. Mainly self-educated. Studied at St. Bartholomew's Hospital, London, for medical qualification without distinction. M.R.C.S., 1880, and L.S.A., 1881.

IV. Professional Career (but for literary and other works besides medicine, see Bibliography):

(a) Transatlantic Ship's Surgeon, 1880–1881.

(b) Entered Indian Medical Service at Madras, April 2, 1881, as Surgeon-Major, a rank he still held when he resigned in 1899.

(c) Began the serious study of Malaria in 1892 (see Bibliography).

(d) Won Parkes Memorial gold medal and prize (triennial) for Essay on Malaria, 1895.

(e) Retired from Indian Medical Service, 31 July 1899.

(f) Lecturer (later "Professor") in Liverpool School of Tropical Medicine, 1899–1914.

(g) Between 1899 and 1929 organized and directed numerous expeditions to tropical and semi-tropical malarious countries, including Sierra Leone, West Africa, Mauritius, Suez Canal, Spain, Cyprus, Greece, Ceylon.

(h) Came to London (while retaining his Liverpool post) in 1912 as physician for tropical diseases to King's College Hospital.

(i) In 1908 took a commission as major in the Territorial (voluntary) Force of the R.A.M.C. (Royal Army Medical Corps), later Lt. Col. on staff of 1st. Western General Hospital, Liverpool.

(j) In 1914, after beginning of the first World War, appointed Consulting Physician for tropical diseases to the base hospitals for Indian Troops in England.

(k) In July, 1915, sent to Alexandria to conduct inquiry into prevalence of dysentery in the Army in the Dardanelles.

(l) Feb. 1917, appointed Consultant for Malaria to the War Office, and same year sent out to Salonika on a malaria inquiry.

(m) In 1919 demobilized with rank of Colonel, and became consultant for malaria to Ministry of Pensions.

(n) Numerous Honours included Parkes Gold Medal, etc., (see (d) and: Silver Medal of London Society of Arts, 1901, Hon. Fellowship of Royal College of Surgeons, England; Fellowship of the Royal Society—both 1901.

1902:—C.B. (Companion of the Bath); Cameron prize of Edinburgh University; Nobel Prize in medicine.

1903:—Barclay bronze medal of Asiatic Society of Bengal.

1904:—D.Sc. degree from Trinity College, Dublin.

1906:—Officer of the Order of Leopold II of Belgium, and L.L.D. of Aberdeen University.

1909:—Knighted by the King; received D.Sc. of Leeds University; given Royal Gold Medal of Royal Society.

1910:—Received degree of M.D., Stockholm; the K.C.B. (Knight Commander of the Bath).

1912:—M.D. of Athens.

1913:—Officier de L'Instruction Publique, France.

1914:—Bisset-Hawkins Gold Medal of Royal College Physicians, London.

1915:—Freeman of the Society of Apothecaries.

1922:—Elected a Member under Rule II (scientific distinction) of the Athenaeum Club, London.

1923:—Albert Medal of the Society of Arts.
1928:—Gold Medal of the Royal Institute of Public Health.
1929:—Manson Memorial Gold Medal, and Gold Medal of the West London Medico-Chirurgical Society.

Some thirty societies have, from time to time, conferred on Ross their membership or fellowship—the term varies—in 1901 the Medical Society of the Dutch Indies, Batavia; in 1902 the Medical Society of Ghent; in 1903 the Societas Medicana Svecana, the Royal Physiographic Society of London, the Manilla Medical Society, the American Society of Tropical Medicine, and the Paris Society of Tropical Medicine and Hygiene; in 1904 the Lancashire and Chesire Entomological Society, and the Paris Academy of Medicine (foreign correspondent); in 1906 the Malaria Society of Greece; in 1907 the Medical Society of Turin (foreign correspondent); in 1908 the Paris Society of Exotic Pathology, the College of Physicians, Philadelphia, the Medical Society of Mauritius (honorary president), and the Society of Tropical Medicine, Cuba; from 1909 to 1911 he was president of the Society of Tropical Medicine, London; the Russia Malaria Commission and the Imperial Military Academy, St. Petersburg, in 1912; the Harveian Society of London and the Institute of Hygiene, London, in 1913; the Society of Sciences, Upsala, and the Biological Society of Belgium in 1919; the School of Tropical Medicine, Brussels, in 1920; the Royal Society of Edinburgh and the Paris Academy of Medicine (Associate) in 1920; the Medical Society of Turin (Fellow), the Royal Society of Literature, London, in 1922.

V. Bibliography (Selected Works)

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1894:—The Third Element of the Blood and the Malaria Parasite, and a List of Natural Appearances in the Blood which have been mistaken for Forms of the Malaria Parasite. *Indian Medical Gazette*, 1894.

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Dr. Manson's Mosquito-Malaria Theory. Indian Medical Gazette, July, 1896.

1897:—On a Condition Necessary to the Transformation of the Malaria Crescent. *British Medical Journal*, Jan. 30, 1897. Further Observations. South Indian Branch, B.M.A., July, 1897, and *Indian Medical Gazette*, Jan. 1898. (Disproves Bignami's contention published in Rome, *Policlinico*, July 15, 1896, and *Lancet*, London, that the flagellate bodies were dying forms.

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(NOTE: Ross's unpublished works include The Emigrants, an unfinished novel; several short stories, two of which have been posthumously printed, and some fragmentary essays. Ross also left MSS of his musical compositions.)

TEMPORARY DEWATERING IN APPLYING DEEPENING AND FILLING TO PERMANENTLY ELIMINATE MOSQUITO BREEDING AREAS IN TENNESSEE VALLEY AUTHORITY IMPOUNDED RESERVOIRS[1]

BENJAMIN R. SIMMS AND CALVIN C. KIKER

Health and Safety Department, Tennessee Valley Authority, Chattanooga, Tenn. and Wilson Dam, Ala.

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The Tennessee Valley Authority is pursuing a program of shoreline improvement for permanent elimination of the major anopheline mosquito breeding areas along the margins of the reservoirs impounded on the Tennessee River. The initial work on this program was done in the Kentucky Reservoir prior to impoundage, and was reported by Bishop and Gartrell (1943) and Gartrell and Kiker (1946). In the main, this program has consisted of diking and dewatering and deepening and filling to permanently eliminate or provide positive control of anopheline mosquito breeding in the larger and more important marginal areas of the reservoirs. The success of the overall permanent shoreline improvement program in the Kentucky Reservoir led to its expansion to embrace similar problems in the flat marginal topography of other main river reservoirs. The aerial mapping work to obtain necessary topography for planning the permanent improvement was reported by Kiker and Morris (1946).

The upper Valley reservoirs are located in relatively steep topography where water level management and limited use of conventional antilarval measures are proving quite satisfactory both as to effectiveness and cost; hence, permanent shoreline improvement is not indicated.

DIKING AND DEWATERING

Permanent diking and dewatering has application to the larger areas having limited watershed and topography favorable for diking. A disadvantage of this method is that continuing annual expenditures are required for maintenance of the interior drainage system and operation of the pumping plant. In addition, leaks or seepage, such as developed in a limited portion of three of the eight projects in the Kentucky Reservoir, may develop to increase pumping costs.

DEEPENING AND FILLING

Due to a very thorough pre-impoundage reservoir preparation, annual shoreline maintenance after impoundage and improvements in water level management practices, growth invasion in the main river reservoirs has been reduced from an original vertical distance of 3 to 5 feet to a present zone of 2 feet or less. Since the production

[1] This paper was presented at the XXX annual meeting of the National Malaria Society, Atlanta, Georgia, December 2, 1947.

of *A. quadrimaculatus* mosquitoes is closely associated with growth invasion, consideration can now be given to the general use of deepening and filling where formerly, due to the greater depth of section, it was not economically sound except in unusual circumstances. Deepening and filling is now being recommended where it is prac-

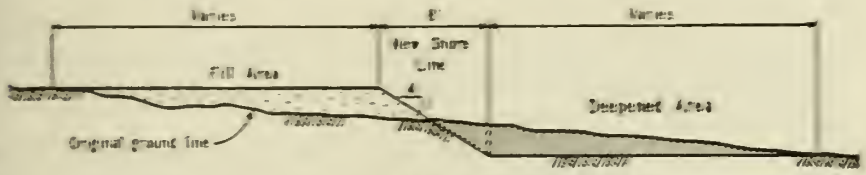

FIG. 1

FIG. 2

tical of application and the savings in repetitive malaria control measures would amortize the cost of the initial construction in a reasonable period of time.

The deepening and filling procedure involves the elimination of shallow problem areas by deepening the lower portion of the area and utilizing the earth removed to fill the upper portion, thus creating a new and relatively steep shoreline which does not offer a significant mosquito breeding potential. Figures 1 and 2 illustrate the

principle involved. Where favorable water level management is assured, the deepening and filling procedure practically eliminates the need for repetitive mosquito control measures.

The following practices are considered fundamental in the design of the deepening and filling projects:

1. The lower limit of the deepened area should extend to or below the lower limit of significant growth invasion. All sources of information should be utilized in establishing or forecasting the limits of growth invasion significant in mosquito production.
2. The upper limit of the filled area should extend slightly above the normal maximum summer pool level (0.3 foot is being used at present in TVA Reservoirs.)
3. The proposed new shoreline should be gently sloping and as straight as practicable, consisting of straight lines and sweeping curves, without involving excessive cuts, fills, or hauls. Narrow embayments should be eliminated insofar as practicable since wind and wave action along the new shoreline tends to inhibit mosquito production. Limited erosion or "flattening out" of the new shoreline under these conditions of exposure is not objectionable.

In theory, deepening and filling is designed so that the amount of the excavation removed to fulfill requirement No. 1 will balance the amount of fill needed to satisfy requirement No. 2. A theoretical balance is obtained rarely, if ever, due to practical construction considerations involved in meeting requirement No. 3 which might include some excavation to waste, borrowing above the upper contour to reduce the length of haul, filling below the mid-contour, or deepening above the mid-contour. Earth compaction allowance, which will vary with the soil type, moisture content, and type of equipment, should be made in designing the projects. Compaction losses on Tennessee Valley Authority projects have varied considerably.

Several types of earth-moving machines, working either singly or in combination, are being used for deepening and filling operations in the Tennessee Valley Authority reservoirs. These include the RD-6 and RD-8 bulldozers, 6 and 16 cubic yard pans or carryall scrapers, draglines and a cable-operated dragscraper. The latter was employed experimentally for excavating in boggy areas where the soil would not support caterpillar tractor equipment. This equipment is essentially a dragscraper motivated by tractor-powered hoisting drums with cable anchorage to a movable tail tower. The method has some use on Mississippi River levee construction, water-filled gravel pit excavating, etc. Where only bulldozers are being used, hauls are limited to 100 to 150 feet. Draglines have been used to effect the excavation in wet ground situations but the range of such machines is quite limited.

TEMPORARY DEWATERING TO FACILITATE DEEPENING AND FILLING OPERATIONS

Temporary diking and dewatering was employed very successfully during the season of 1947 in the Chickamauga Reservoir to facilitate deepening and filling operations during periods of high lake level. Under conditions of a minimum of temporary dike construction and limited watershed, the run-off pumping costs have amounted to as little as 5 per cent of the total cost of the shoreline improvement.

In the initial use of temporary dewatering it was thought that the principal con-

trolling factor in design of the pumping installation should be the size of the watershed and total run-off. From the experience gained during the summer of 1947 it is now believed that the principal factor in pump design should be the time or amount of pumping required to dewater the area. This is based on an assumption of construction during the most preferable working period (May–December) and the relative infrequency of heavy rains which would completely fill the dewatered area. When such rains occur during the construction period, the dike would be breached and as much water as possible passed into the lake by gravity. When the principal flow has passed, the dike would be repaired and dewatering initiated. During a major portion of the period, Tennessee Valley Authority lakes would be expected to be at some elevation lower than the maximum summer elevation, thus permitting passage of a portion of the run-off by gravity which would limit the amount of pumping required for keeping the area dewatered. Under ordinary conditions, special outlet drainage structures, such as pipes or culverts, are not required.

In several of the main river reservoirs a low water period occurs normally in the fall of the year, thus permitting an opportunity for application of the deepening and filling procedure without diking and temporary dewatering. However, in one of the reservoirs the maximum drawdown of the water level is only 2 feet and in others the low water period is quite uncertain, thus making the application of the deepening and filling procedure with conventional equipment impossible or uncertain without use of temporary dewatering and, at best, the working period would be of relatively short duration (October–December).

The Washington Ferry slough was the first temporary dewatering project undertaken in the Chickamauga Reservoir. The dewatering was abnormally expensive due to the use of a gravity outlet through the dike and a make-shift pump installation pending shipment of the low head, high volume impeller-type pump specially designed for this type of work. The dike was installed before the lake reached normal maximum elevation; hence pumping was required only for handling run-off from the watershed. The deepening and filling was done during the period April 7 to June 18, 1947. The deepening and filling involved a total section 2 feet in depth which embraced 30 acres of mosquito breeding area. Rainfall averaged 2.7 inches per month during the period, of which 0.4 inch per month run-off from the 160-acre watershed was required to be pumped. The following table reflects the direct field costs accumulated on the operations.

Washington Ferry Slough Project

Costs	
18,000 cu. yds. excavation deepening & filling @ 21¢ or 660 machine hours @ $5.75	$3,800
600 cu. yds. excavation per acre	
Dike Construction—150 cu. yds. earth excavation, 470 B.F. lumber	390
Pump Installation	160
Pump Operation—165 hrs. @ $2.18	360
General Expense—Field Supervision, Grade Checking, Auto Mileage, etc.	1,090
Total Cost—30 Ac. @ $194 per acre	$5,800

Thus the total cost for permanent elimination of the mosquito breeding problem in this area amounted to $194.00 per acre. The work will permit an annual saving of $21.00 per acre for repetitive antilarval measures. At 3 per cent interest on the capital investment, the saving will permit amortization in a period of about 11 years, which appears to be ample financial justification for the improvement. The cost of temporary dewatering was approximately 16 per cent of the total cost of the project, which was abnormally high due to reasons indicated previously. It is of interest to observe that the lessee of the marginal land bordering this project sowed the 14-acre top of the new fill section to canary grass and lespedeza in the establishment of a permanent pasture. The creating of usable and valuable marginal land as a by-product of the deepening and filling operation is not being taken into account in the financial justification of such projects but it is important that the improvement

Fig. 3

definitely changes the area from the status of a liability to that of an asset. The most desirable use of the land appears to be for growing pasture grass, hay crops, or timber. Row cropping is objectionable since the tops of the fills are only .3 foot above the normal summer maximum water elevation and water standing in the furrows would result in mosquito production.

The successful outcome of the Washington Ferry Slough project led to undertaking a similar area in the vicinity known as the Locke Bottom project. In the meantime, the larger and more efficient pump had been delivered. It is of the low head high volume turbine type which has been used extensively for agricultural drainage in Florida. Figure 3 shows the pump in operation on the Locke Bottom project dike. This pump delivers 4,000 g.p.m. under a 6 foot static head and is powered by a small utility tractor of 18 h.p. Figure 4 shows the manufacturer's performance graph on this particular pump. Figure 5 shows deepening and filling in progress on the Locke Bottom project, and figure 6 a section of the completed new shoreline. The tem-

porary dike of the Locke Bottom project was closed on June 4, 1947, with the lake ½ foot below the normal summer maximum elevation. Pumping was initiated on a 24-hour schedule until the area was dewatered on June 9. The deepening and filling

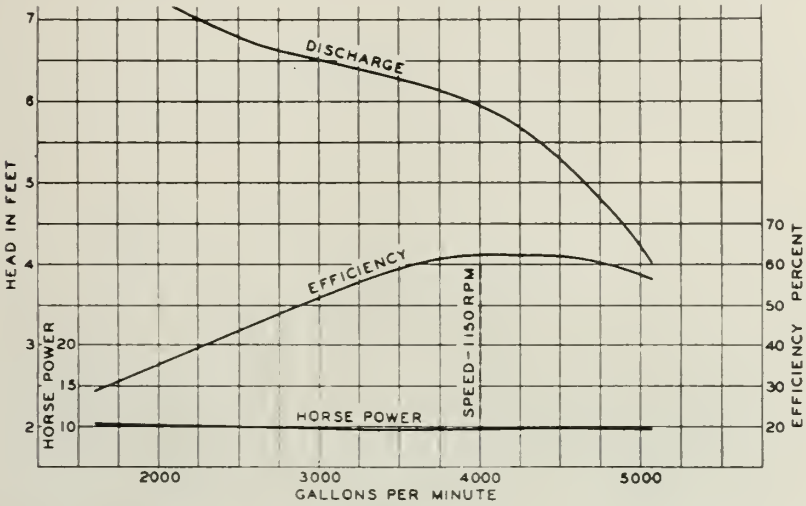

Fig. 4

Fig. 5

was done during the period of June 19–September 15 and embraced a section approximately 2 feet in depth and 65 acres of mosquito breeding area. Rainfall during the period averaged 3.1 inches per month, 0.3 inch of which represented run-off estimated to have required pumping in keeping the area dewatered. The following table reports the direct field cost of the operation.

Locke Bottom Project

Costs	
45,600 cu. yd. excavation deepening & filling @ 18¢ or 1200 machine hours @ $6.90	$8,280
700 cu. yds. excavation per acre	
Dike Construction—500 cu. yds. earth excavation	120
Pump Installation	80
Pump Operation—110 hrs. @ $2.37	260
General Expense—Field Supervision, Grade Checking, Auto Mileage, etc.	1,260
Total Cost—65 Ac. @ $154 per acre	$10,000

It will be observed that the overall cost per acre for eliminating this mosquito breeding area was $154 which has resulted in an annual saving of $21.00 per acre for

Fig. 6

routine annual antilarval measures. Based on a rate of 3 per cent interest for the capital investment, this project would be amortized in a period of about 8 years. The additional cost for providing diking and dewatering in order to extend the work period was $460, which amounts to a little more than one cent per cubic yard of earth moved, or $4\frac{1}{2}$ per cent of the total cost of the project. It is to be noted that bulldozer machine rental advanced approximately 20 per cent at the time of beginning work on the Locke Bottom project.

Immediately upon completion of the Locke Bottom project the equipment was moved to an additional one referred to as Bullfrog Creek. This area of 56 acres constitutes the most difficult and expensive mosquito control problem on the Chickamauga Reservoir. Spring-fed seepage occurs over a large portion of the area, thus the ground would not support caterpillar type earth-moving equipment in this condition. The plan provides for a combination of deepening and filling, borrowing to fill and excavating to waste below the growth invasion zone. Ground conditions

were improved by construction of a drainage ditch from a temporary low head levee to the upper reaches of the spring-fed area. The run-off was disposed of over the dike into the lake, which was some 3½ feet below normal elevation at the time, by means of the 4,000 g.p.m. low head pump. Construction of the drainage channel to an elevation 5 feet below the lake, combined with adequate pumping of the run-off, effected a lowering of the ground water table and increased the acreage where the deepening and filling could be applied. This project is still under construction but it is evident that the operational data will parallel that assembled for the other projects.

INCIDENTAL PROBLEMS

Wet ground conditions appear to be the most serious obstacle to application of the deepening and filling procedure. Frequently, drying out can be facilitated by removing the vegetation and constructing drains to lower the ground water level. Where sheet seepage occurs either from natural sources or as a result of water from the lake finding its way into the temporarily diked area through underlying pervious strata, it will be difficult to use conventional caterpillar equipment. Such conditions have been encountered to a limited extent and are being met by use of such equipment as the dragscraper or dragline, or in case of lake seepage, doing the work without temporary diking and dewatering when the lake is at a low elevation. Another method has been complete filling from borrow above the contour but this increases the haul and yardage. Theoretically, complete filling requires four times the yardage required for a balanced cut and fill. For this reason, dredging, which would require complete filling, has not been employed.

For practical reasons the top of the fills and bottoms of the cuts are set on a flat grade. The shallow depressions which may develop on the top of the fill can be easily drained by small ditches. Where natural springs or seepage areas occur in the fill area, it is important that they be drained by open ditch, tile, or conventional french drains. Adequate drainage should be provided for the natural ground back of the fills so no impoundage will occur. The natural drains through the fills should be of ample and uniform sections in order to limit bank erosion to a minimum.

CONCLUSION

The experience to date in the use of temporary diking and dewatering to facilitate the application of permanent shoreline improvement in reservoirs which have been impounded has been quite favorable, both from the standpoint of cost and in prolonging the working period. It has now been adopted as one of the accepted methods in the Authority's shoreline improvement program to permanently eliminate the need for repetitive malaria control measures in reservoirs on the Tennessee River.

SUMMARY

Reference is made to the Authority's initial use of shoreline improvement in the Kentucky Reservoir to permanently eliminate major mosquito breeding areas and the expansion of the program to embrace other main river reservoirs offering a malaria problem.

A detailed account is given of the initial use of temporary diking and dewatering to facilitate the deepening and filling procedure in the Chickamauga Reservoir during the season of 1947. Data accumulated on the work indicate that the method may be applied to an overall section two feet in depth for from 600 to 700 cubic yards of excavation per acre of mosquito breeding area, or a cost of $150–$200 per acre, only 5–10 per cent of which would be required for temporary diking and dewatering, utilizing specially designed pumps of high capacity and efficiency operating under heads around 6 feet.

Described are some of the construction problems encountered as a result of seepage and wet ground conditions and how such problems are being met. Conclusion is reached that temporary diking and dewatering will be used extensively in facilitating application of the deepening and filling procedure to eliminate major mosquito breeding areas in TVA reservoirs offering a malaria problem. Further, that there appears to be ample justification for the work since the savings in annual cost of repetitive antilarval measures will amortize the capital investment in 8–12 years.

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DURATION OF IMMUNITY TO MALARIA (*PLASMODIUM CATHEMERIUM*) IN THE CANARY[1]

WENDELL D. GINGRICH

University of Texas Medical School, Galveston, Texas

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The duration of immunity in avian malaria has not been studied previously for lack of agents to eradicate infection completely. In monkeys the duration of immunity to *Plasmodium knowlesi* following cure with sulfonamides was studied by Coggeshall (1938) and Maier and Coggeshall (1944) and may be said to be approximately one year. Since radical cure of established infections of *P. cathemerium* in canaries has been accomplished (Gingrich et al., 1947), the present study was made by observing the course of infection in cured birds following standard inocula of asexual forms of the 3H2-1 strain (Gingrich and Schoch, 1947).

At monthly intervals up to eight months, intravenous inoculations of 10 million or 0.5 million parasites were given to canaries previously treated with curative drugs (SN 8557 or SN 13,421 or combinations of these with SN 13,276; see Wiselogle, 1946) during the latent phase of blood-induced infections. Birds with latent infections and normal ones inoculated at the same time served as the two types of controls, immune and susceptible. It had been planned originally to have a regular number of cured birds at the selected intervals all treated similarly and at the same time, but circumstances did not permit the anticipated uniformity. Nevertheless, the available data are quite satisfactory and may be summarized as to types of infections studied at the intervals indicated in table 1. The experimental techniques and the criteria of cure used in this work are the same as those described by us previously (Gingrich et al., 1947). It may be repeated here that our criteria of cure are two negative blood transfers at intervals of one and two months after treatment, followed by susceptibility to reinoculation. Obviously the negative transfers requirement could not be satisfied for the tests of the first two months reported here and the results of the reinoculations are those described below.

One month following treatment a group of 10 birds together with 5 in latent phase of infection and 5 normal ones each received intravenous inoculation of 10 million parasites. Daily blood examinations revealed an increased parasitemia for two or three days in 8 of the treated birds although lower than that of the normal birds, and all were negative by the sixth day. Two birds of this group reacted in a manner indistinguishable from the latent group which were negative in 24 to 72 hours after inoculation.

[1] The work described in this paper was begun under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Texas. It was continued under a grant from the John and Mary R. Markle Foundation and completed under a Grant-in-Aid from the National Institute of Health of the U. S. Public Health Service. The author acknowledges the assistance of Miss Marjorie Schwab and Mrs. Margaret O'Neal.

At the two month interval following treatment 5 birds received 10×10^6 parasites intravenously and 5 received 0.5×10^6. For each of these groups 5 immune (latent) and 5 normal birds received similar inoculations. The results for each group are represented in figures 1 and 4. (Only two susceptible controls are included in the graphs in order to show the others to better advantage.) In all of the experimental birds which received 10×10^6 parasites there was a definite increase in parasitemia during the first two days after which time the patent parasitemia disappeared by the 3rd to 6th days. In fact, the rate of increase in some birds does not appear to be greatly different from that in the susceptible controls. Four of the five immune birds showed decreasing parasitemia with negative blood findings within 3 or 4 days.

TABLE 1

NO. MONTHS AFTER CURE	NO. PARASITES INOCULATED	NUMBER OF BIRDS INOCULATED		
		Cured	Latent	Normal
1	10×10^6	10	5	5
2	10×10^6	5	5	5
	0.5×10^6	5	5	5
3	10×10^6	5	5	5
	0.5×10^6	5	5	5
4	10×10^6	5	5	5
	0.5×10^6	5	5	5
5	10×10^6	63*	13	9
6	10×10^6	5	5	5
	0.5×10^6	5	5	5
8	0.5×10^6	6	5	5

* 11 of these were sporozoite-induced 3H2-1 and 19 were blood-induced 3C strain.

The fifth bird of this group was unusual in that it did not present negative blood examination until the 6th day. In the cured birds which received 0.5×10^6 parasites the parasitemia increased even longer (about 3 days) than with the larger inoculum. Again, the parasites were reduced to negative blood examinations far more promptly (5 to 7 days) than in controls. And in the immune birds the blood was negative within 3 days. The results at the three months interval show a trend toward those of a month later.

Four months following curative treatment the same procedure was repeated with similar groups of canaries and the results are illustrated in figures 2 and 5. With the larger inoculum the parasitemia in the cured birds increased for 2 to 5 days and blood examinations were negative on the 4th to 8th days after inoculation. Here the difference in the experimental animals between both types of controls, immune and susceptible, is quite definite. With the smaller inoculum of 0.5×10^6 the pattern of

infection runs over into that in normal canaries. In this instance the parasites increased in cured birds over a period of 3 to 4 days, and were no longer demonstrable in blood films after 5 to 8 days.

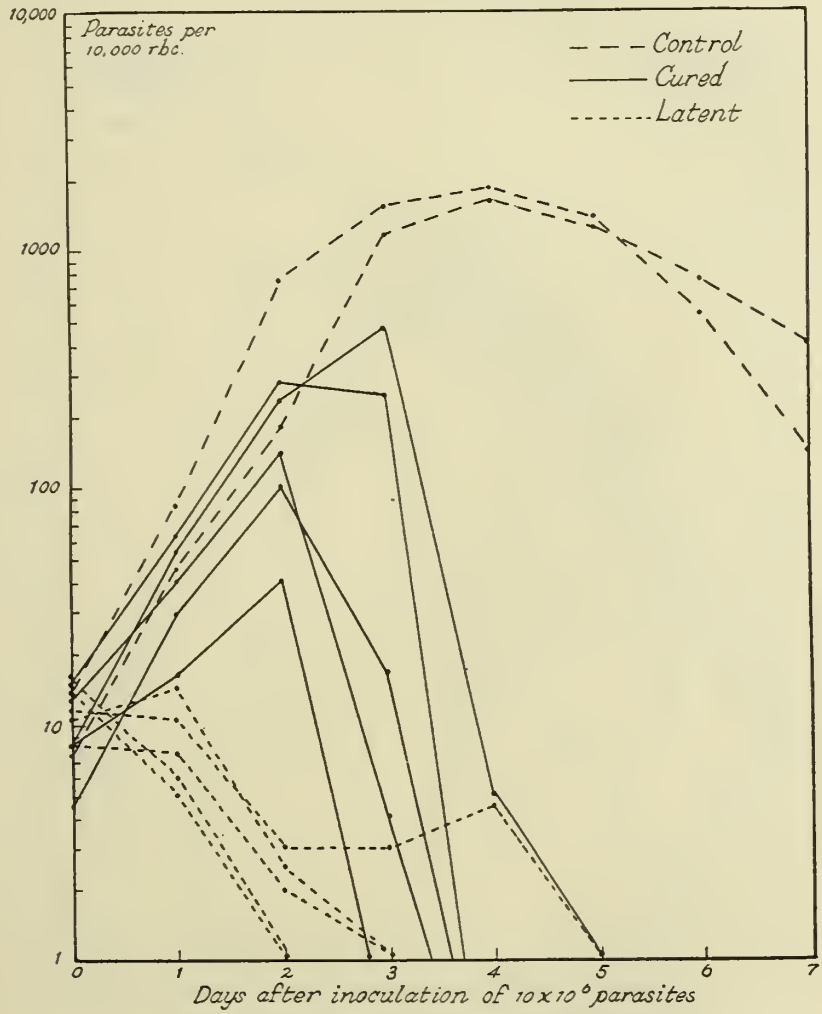

FIG. 1. Two months after cure

For the fifth month a large number of birds were examined at different times, all inoculated with 10×10^6 parasites. In no instance was there any difficulty in distinguishing the infections in cured birds from those in immune birds because of the increase in parasitemia for 3 to 5 days. In the 11 birds which had sporozoite-induced infections the parasites increased for 3 to 4 days to 128–984 parasites per 10,000 rbc. And in the 19 birds which had the virulent blood-induced 3C strain they increased for 3 to 5 days to 189–1143 parasites per 10,000 rbc.

Six months after cure 5 birds each were inoculated with 10×10^6 and 0.5×10^6

parasites along with appropriate controls as listed in table 1. The results are shown in figures 3 and 6. Following inoculation of 10 million parasites the infections in cured birds reached almost as high a peak on the average as in controls. Blood examinations, however, became negative in 6 to 9 days, significantly earlier than the

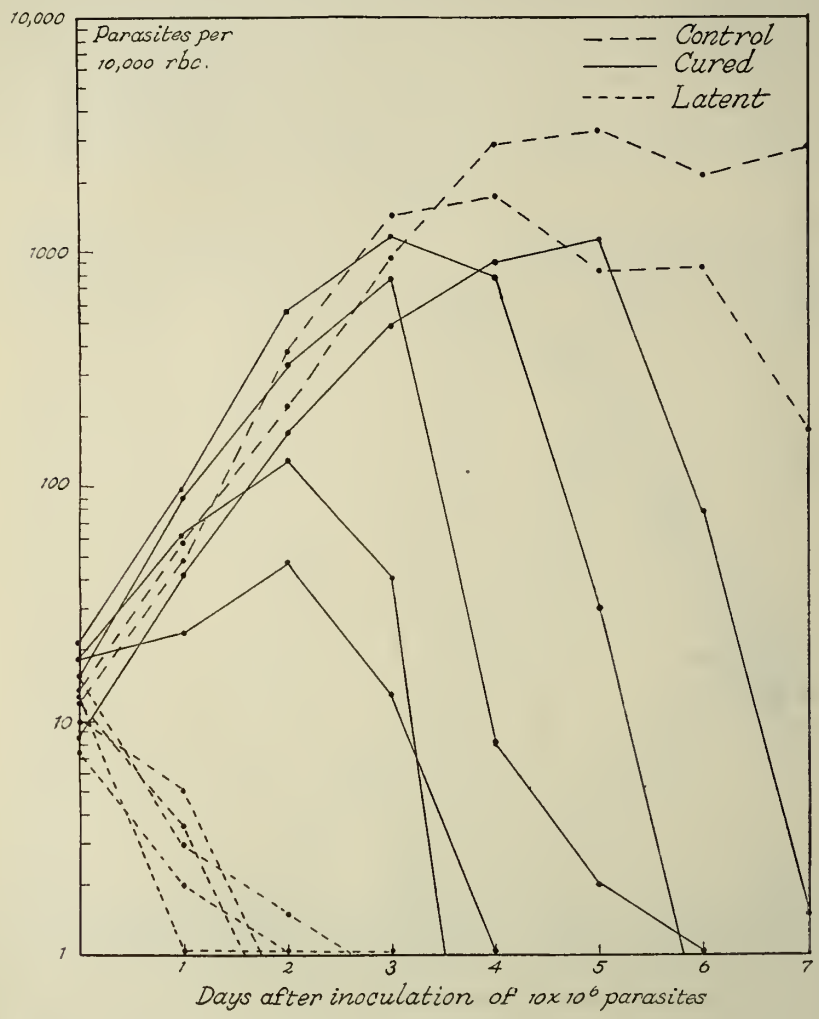

FIG. 2. Four months after cure

10 to 21 days for controls. With half a million parasites inoculated, the average peak of parasitemia in cured birds again almost reached that of normal birds. Examinations of blood films were negative on the 6th to 9th days as compared to the 9th to 14th days for controls.

At eight months following cure six birds were inoculated intravenously with 0.5 $\times$ 10^6 parasites. The resulting infections were similar to those of the susceptible

controls in most respects. The mean parasitemia on the fifth day after inoculation was 1154 per 10,000 rbc., (ranging from 904 to 1420/10,000 rbc.) and 1310 (898 to 1540) per 10,000 rbc., for the controls. Blood examinations were negative for the experimental birds on the 8th to 12th days and on the 10th to 14th days for controls.

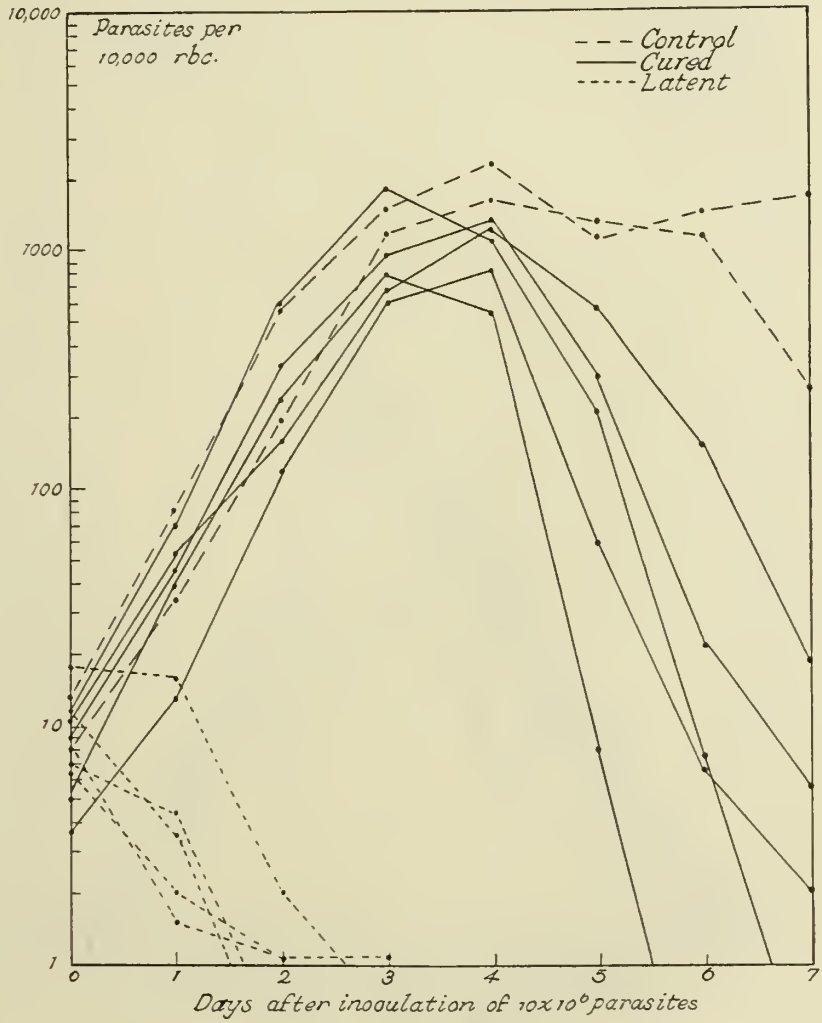

FIG. 3. Six months after cure

There is, therefore, no definite indication of immunity persisting in this group, eight months following cure.

DISCUSSION

The immunity which accompanies latent infection of *Plasmodium cathemerium* in canaries, following recovery from the initial acute attack, was taken as both the

starting point and a basis for comparison in this investigation. It is remarkably efficient (Taliaferro and Taliaferro, 1929) and more nearly uniform than with many other infections.

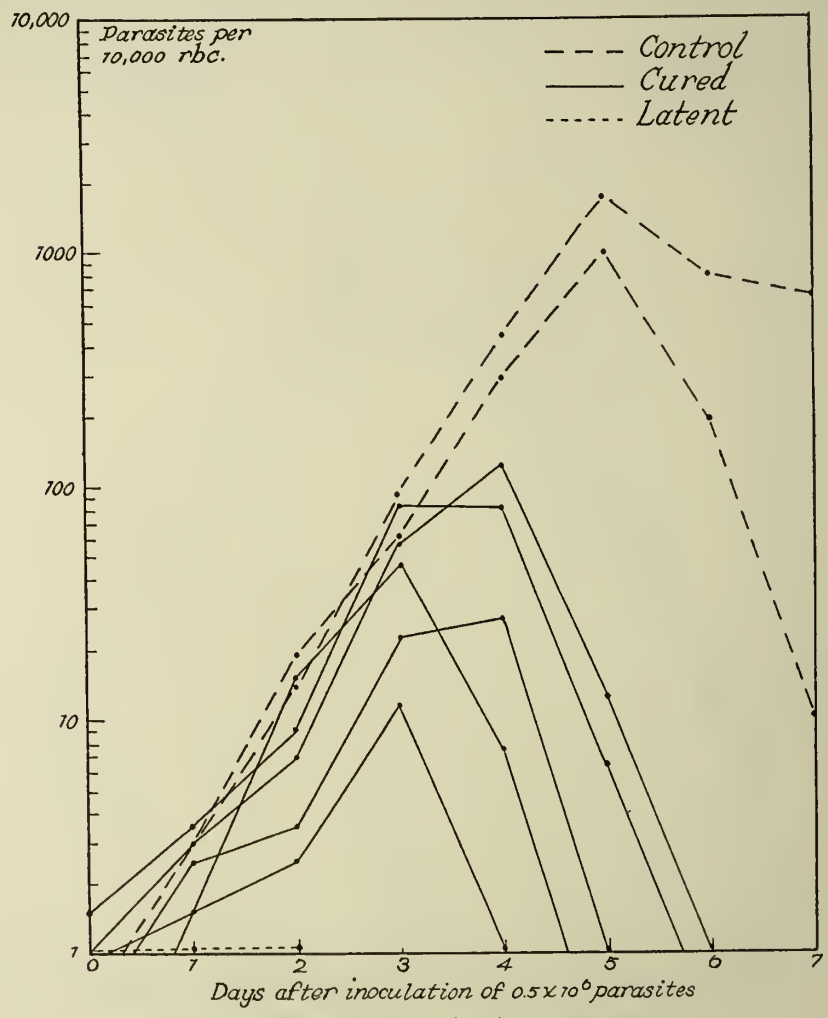

FIG. 4. Two months after cure

Allowing for individual variation, inspection of the graphs reveals an increasing time interval between inoculation and decreasing parasitemia from 2 to 6 months after cure. This is evidently the period of time required for resensitization or for reactivation of the macrophage system. A further point of interest is the rate of increasing parasitemia as shown by the slope in the semi-logarithmic graphs. In some of the cured birds this rate is the same as that of controls for as long as three days after inoculation. The obvious inference is that there is no acquired immunity

operating during this period, even though it is more efficient than in controls when it does begin to manifest itself by the decreasing parasitemia. In others of the cured birds the rate of increasing parasitemia appears to be significantly lower than in controls and these birds recover more rapidly.

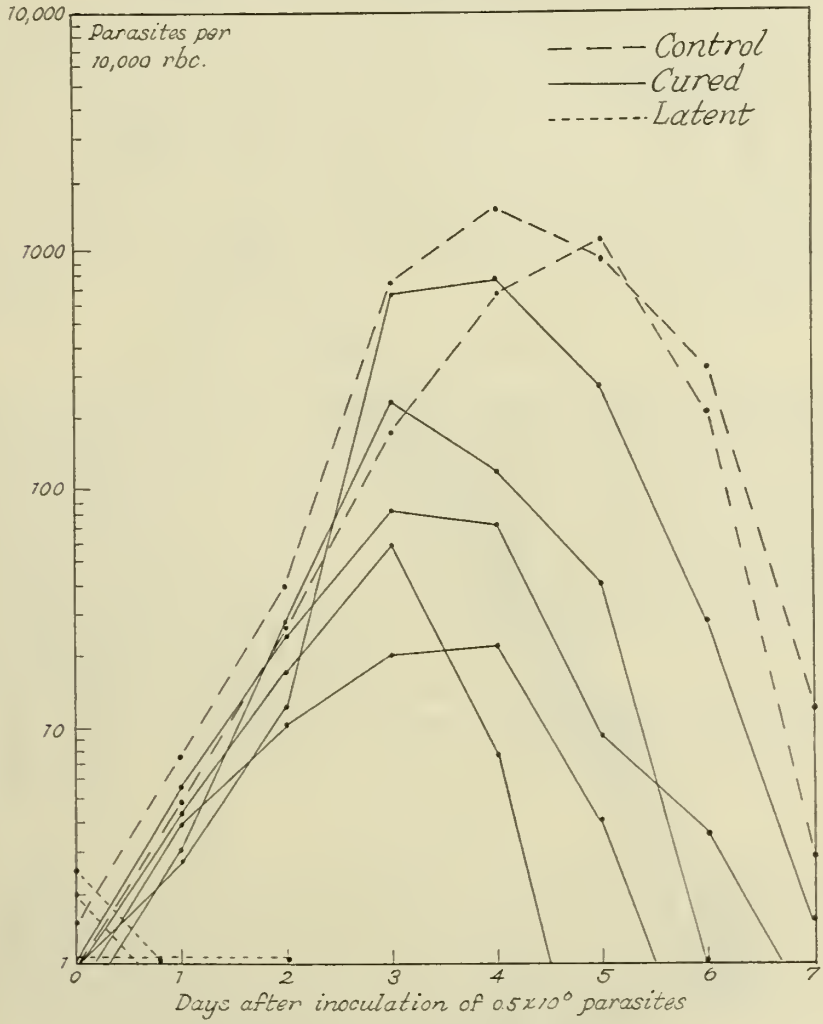

FIG. 5. Four months after cure

Applying the criteria of (1) increasing or decreasing parasitemia following inoculation, (2) the height of parasitemia, and (3) the time for blood examination to become negative, it is evident that a majority of cured birds have lost some degree of acquired resistance even as early as one month after cure, and the loss is progressive thereafter. Although there is obvious individual variation, there appears to be sufficient uniformity to generalize to the effect that a partial immunity remains for

about six months. One practical consideration which may be derived from the progressive and relatively uniform loss of resistance is valuable in testing for cure, following administration of drug. Inoculation of 10×10^6 parasites of this particular strain four months, or later, after treatment can be depended upon to demonstrate cure by the resulting increase in parasitemia for three or more days.

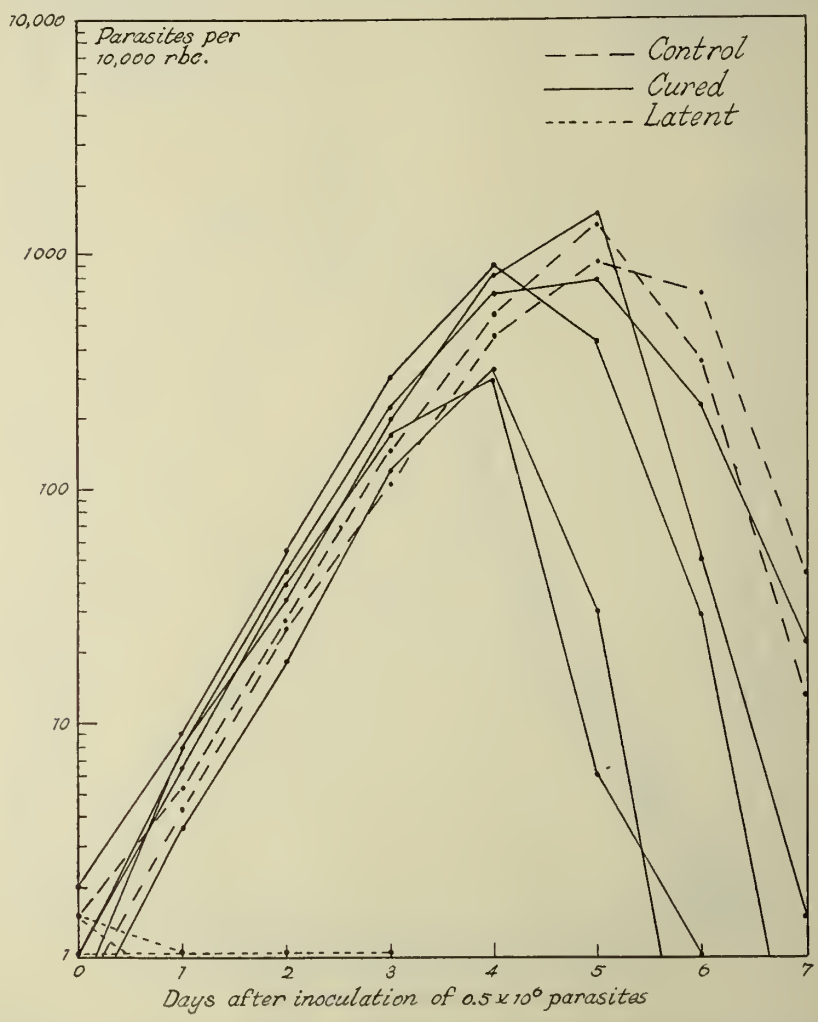

FIG. 6. Six months after cure

The particular means of evaluating immunity used in this study reveals the reaction within the first 24 hours as well as later. It is to be anticipated that other methods may yield results somewhat different. For example, if the inoculation were lethal for 50 per cent of controls, one might use survival rate and arrive at a somewhat different result. Similarly, with sporozoite-induced infections with a prepatent period of five days there is a great deal more time for any partial immunity

to exert its effect and again the result may be different from the round number of six months as in the present study.

To the question as to whether loss of immunity following cure of sporozoite-induced infections differs from that of blood-induced infections the answer is definitely negative. In the birds which had had sporozoite-induced infections, the test inoculation five months after cure resulted in increasing parasitemia for 3 to 4 days. In this same group were 19 birds which had had blood-induced infections of the more virulent 3C strain. They also allowed increasing parasitemia for 3 to 5 days. One may therefore conclude that neither the manner of inducing infection nor the virulence of the strain effect any significant difference in loss of immunity following cure—at least when tested by blood inoculation of the very mild 3H2-1 strain. This may be somewhat surprising since both sporozoite-induced 3H2-1 and blood-induced 3C are almost invariably fatal because of prominent exo-erythrocytic infection, whereas this type of infection has thus far not been detected in blood-induced 3H2-1 infections.

SUMMARY AND CONCLUSIONS

In canaries cured of latent infections of *Plasmodium cathemerium* the progressive loss of acquired immunity was demonstrated following intravenous inoculation of 0.5×10^6 and 10×10^6 asexual parasites (3H2-1 strain) at monthly intervals following curative treatment. As early as one month after cure most of the birds allowed some increase in parasitemia for 2 to 3 days. At four months the loss of resistance is sufficient to permit reinoculation as a test for cure. At six months there remained a small but definite measure of partial immunity as manifested by the slightly lower peak and duration of patent parasitemia. At eight months there was no longer any definite indication of acquired immunity.

The loss of immunity following cure in birds which had originally had sporozoite-induced infections and blood-induced virulent 3C strain infections, was the same at five months as in birds which had originally had blood-induced infections of the mild 3H2-1 strain.

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REACTIONS OBSERVED DURING TREATMENT WITH PENTAQUINE, ADMINISTERED WITH QUINACRINE (ATABRINE), METACHLORIDINE[1] (SN-11,437), AND WITH SULFADIAZINE[2]

JOHN A. ATCHLEY,[3] ERNEST H. YOUNT,[4] JOEL R. HUSTED, THEODORE N. PULLMAN, ALF S. ALVING AND LILLIAN EICHELBERGER

The Malarial Research Unit, Department of Medicine, University of Chicago, Chicago, Illinois

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Pentaquine (SN-13,276) and quinine, when administered concurrently, have been shown to possess curative properties against *Plasmodium vivax* infections (Loeb, et al., 1946; Alving, et al.).

Pentaquine monophosphate when administered alone, at a daily dosage of 80 mgm. (equivalent to 60 mgm. of the base), has considerably less curative effect than an equal amount given concurrently with quinine (Alving, et al.). In order to determine whether potentiation of pentaquine will also result from the concurrent administration of other suppressive antimalarial drugs, regimens were tested incorporating pentaquine with quinacrine (atabrine), chloroquine (SN-7618) (Loeb, et al., 1946), paludrine (Adams, et al., 1945), and metachloridine (SN-11,437) (Pullman, et al.). Sulfadiazine was included in toxicity studies because of its close chemical relationship to SN-11,437.

The clinical testing uncovered no evidence of marked therapeutic synergism except with quinine. During the course of the tests, however, certain toxic reactions were encountered which are of clinical importance. The most serious of these were granulocytopenia and agranulocytosis, which occurred when either SN-11,437 or sulfadiazine was administered concurrently with pentaquine.

PROCEDURES AND RESULTS

The general procedures used have been reported in detail elsewhere (Alving, et al.). The subjects were white male volunteers who gave no history of previous exposure to malarial infections.

[1] Metachloridine is the proprietory name for N′-(5-chloro-2-pyrimidyl) metanilamide, one of a number of metanilamides prepared in the Stanford Laboratories of the American Cyanamide Company.

[2] This investigation was carried out under a grant-in-aide from the United States Public Health Service to the University of Chicago. The investigations would not have been possible except for the enthusiastic coöperation of the inmates and the administrative staff of Stateville Penitentiary. This work was further aided by the participation of Army Medical Officers assigned to the project by the Surgeon-General, U. S. Army.

The authors are indebted to Major Martin D. Young, U.S.P.H.S. and Captain Don E. Eyles, U.S.P.H.S. and the staffs of the U. S. Public Health Service Malaria Research Laboratories at Columbia, South Carolina and Milledgeville, Georgia for supplying *Anopheles quadrimaculatus* mosquitoes infected with the Chesson strain of *vivax* malaria and for technical aid during inoculation of the volunteers.

[3] Captain, M.C., A.U.S.

[4] Lieutenant, M.C., A.U.S.

All subjects received 60 mgm. per day of pentaquine base (80 mgm. of monophosphate salt) in six equal divided doses.

Pentaquine and paludrine. Paludrine was administered concurrently to five patients with malaria. They received a total of 16.8 grams of paludrine acetate in 14 days. The toxicity of pentaquine was not increased when paludrine was administered simultaneously.

Pentaquine and chloroquine. Chloroquine was administered concurrently with pentaquine to five patients with malaria. They received 5.65 grams of chloroquine

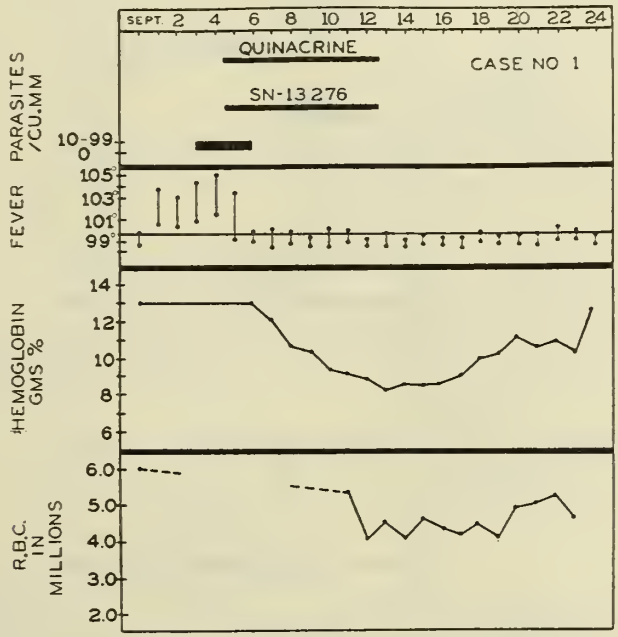

FIG. 1. Chart of patient receiving pentaquine (SN-13,276) and quinacrine concurrently for treatment of an acute attack of *vivax* malaria. The highest and lowest recorded rectal temperatures for each 24-hour period are recorded as dots connected by a vertical line.

diphosphate in 14 days. Chloroquine did not enhance the toxic symptoms due to pentaquine.

Pentaquine and quinacrine. Quinacrine was administered concurrently with pentaquine to five patients with malaria. The four patients who completed a 14-day therapeutic course received 5.0 grams of quinacrine dihydrochloride. A mild hemolytic anemia occured in the fifth patient, an adult Italian male, (fig. 1) shortly after the start of therapy. When treatment was initiated, this patient's hemoglobin began falling from a concentration of 13.0 grams per hundred cubic centimeters to 8.3 grams per hundred cubic centimeters on the eighth day of drug administration. At this time, both drugs were discontinued and ferrous sulfate treatment begun. There followed a steady rise in hemoglobin levels to normal. This patient had received a total of 3.2 grams quinacrine dihydrochloride at the time the therapy was discontinued. During a subsequent relapse the patient did not develop hemolytic

anemia on the administration of 60 mgm. of pentaquine base and 2 grams of quinine sulfate daily for 14 days.

Pentaquine and SN-11,437. SN-11,437 was administered concurrently with pentaquine to five patients with malaria. Four subjects received from 20 to 22 grams of SN-11,437 over a 14-day period, which produced plasma drug concentrations between 12 and 13 mgm. per hundred cubic centimeters. The fifth had received 2 grams when drug was withdrawn on the second day. The plasma concentration of SN-11,437 was 10 mgm. per hundred cubic centimeters.

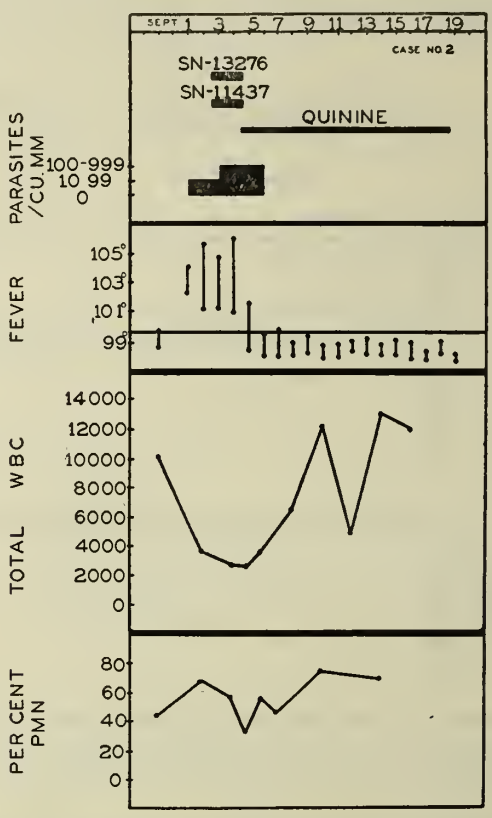

FIG. 2. Chart of patient receiving pentaquine (SN-13,276) and SN-11,437 concurrently for treatment of an acute attack of *vivax* malaria. Since parasites were still present in the peripheral blood when pentaquine and SN-11,437 were discontinued, quinine was used to suppress the malarial attack.

The clinical course of the patient in whom treatment was stopped is shown in figure 2. Before the onset of malaria, this volunteer had a white blood count of 10,100 cells per cubic millimeter; the total polymorphonuclear leukocytes being 4,700. Although the white count fell to 3,700 during clinical malaria, the fall in polymorphonuclear leukocytes was no greater than is commonly seen during the disease, the total being 2,500 before treatment was begun. On the fourth day of drug administration, however, the polymorphonuclear leukocytes numbered only

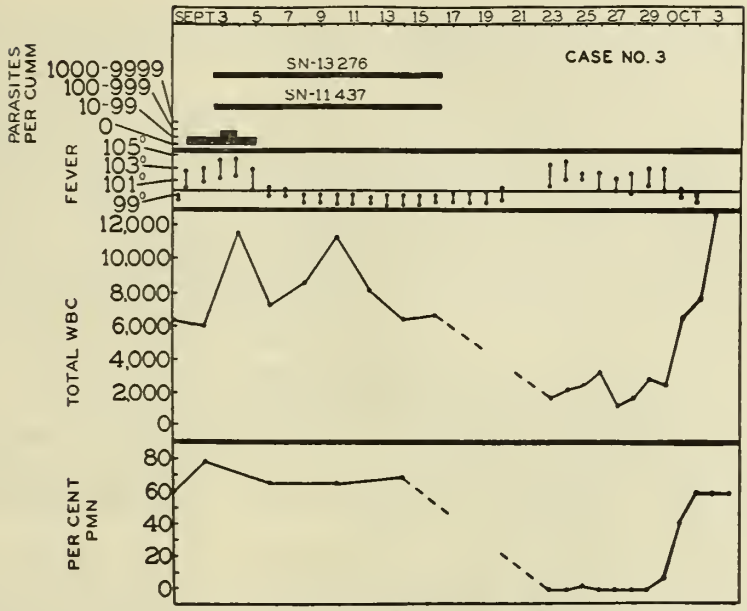

FIG. 3. Chart of patient receiving pentaquine (SN-13,276) and SN-11,437 concurrently for treatment of an acute attack of *vivax* malaria. The granulocytopenia was noted a week after antimalarial therapy had been terminated.

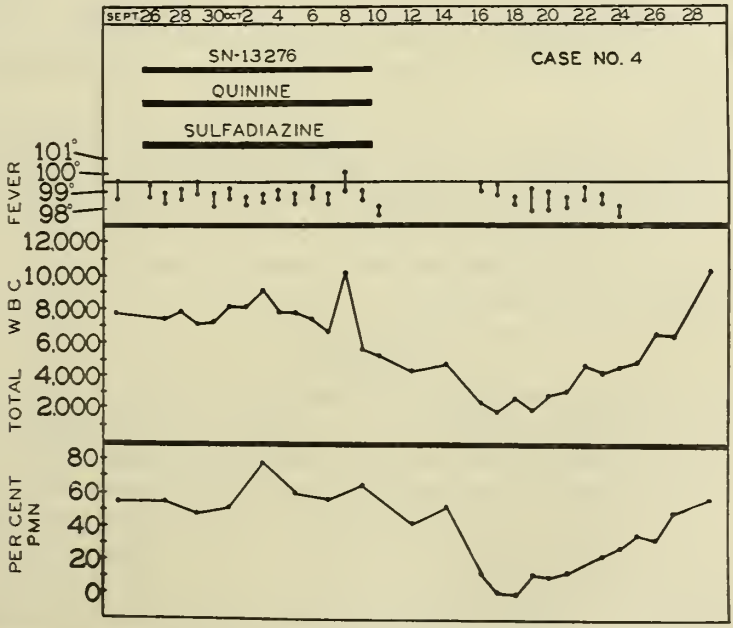

FIG. 4. Chart of a subject without malaria receiving sulfadiazine concurrently with a course of pentaquine (SN-13,276) and quinine. Granulocytopenia began to develop at the end of the two-week course of medication and agranulocytosis appeared a week after drug administration had been terminated.

620, or 24 per cent of the total white blood cells. At this time, both drugs were discontinued and quinine was substituted for control of the clinical attack. There followed a rapid return to normal values.

The other patient in whom a severe reaction occurred (fig. 3) showed no unusual toxicity during the two-week course of drug, but later developed agranulocytosis. No white blood counts were obtained immediately after termination of treatment. On the seventh day following discontinuance of drugs, the patient was admitted to the hospital with a white blood count of 1,700 per cubic millimeter. No polymorphonuclear leukocytes were observed. Despite penicillin, pentnucleotide, pyridoxine, folic acid administration, and daily transfusions, less than two per cent polymorphonuclear leukocytes were found in the blood smear for one week. At the end of this time, both the total white count and the number of granulocytes began to rise, reaching normal levels within another week.

Pentaquine, sulfadiazine and quinine. Four volunteers without malaria were given sulfadiazine (5 grams the first day and 4 grams daily thereafter) along with the two week course of concurrent pentaquine and quinine (60 mgm. of pentaquine base and 2.0 grams of quinine sulfate daily).

One of the volunteers developed agranulocytosis (fig. 4). The white blood count, although normal at the end of medication, began to fall immediately thereafter, reaching 1,800 per cubic millimeter with no polymorphonuclear leukocytes on the eighth day after cessation of drugs. On the seventh day after drugs were discontinued, while the white blood count was falling, pentnucleotide, folic acid, pyridoxine, and penicillin treatment were initiated. Three days later the white blood count and number of granulocytes began to rise and soon attained normal levels. At no time was this man clinically as ill as the patient with agranulocytosis following SN-11,437 and pentaquine.

Effect of concurrent drugs on the concentration of pentaquine in the blood plasma. The concurrent administration of either paludrine or quinacrine caused a marked increase in the concentration of pentaquine in the plasma, as measured by coupling with diazonium salts (Brodie, et al., 1947). Simultaneous administration of SN-11,437 caused a marked reduction, and sulfadiazine a moderate decrease in the concentration of pentaquine in the plasma.

DISCUSSION

It is apparent (table 1) that the reactions noted were not due directly to the concentration of pentaquine in the plasma, because paludrine, which increased the plasma concentration most markedly, did not enhance the toxicity of pentaquine; while the administration of both SN-11,437 and sulfadiazine, which decreased the concentration, was accompanied by toxic manifestations. These findings are compatible with the hypothesis that the toxic reactions, as well as the therapeutic effect, may be attributed to metabolic products of the drug.

The fact that the average methemoglobinemia produced by the pentaquine-quinacrine was approximately three times as severe as that produced by pentaquine in combination with quinine must be considered evidence that quinacrine increases

the toxicity of pentaquine (table 1). Although the occurrence of hemolytic anemia in one of five patients treated with the pentaquine-quinacrine regimen does not constitute conclusive proof that this combination causes hemolytic anemia more frequently than does pentaquine-quinine therapy, a consideration of the subsequent course makes this probable. Hemolytic anemia did not develop during the concurrent administration of pentaquine and quinine in a later relapse. While pentaquine can produce acute hemolytic anemia in white subjects, it does so rarely. Acute hemolytic anemia has only been observed in one of 186 white volunteers to whom 60 mgm. of pentaquine base or more were administered (with or without quinine) during the clinical testing program at Stateville Penitentiary over the last three years.

TABLE 1

Average methemoglobinemia and average concentration of pentaquine (SN-13,276) in plasma, when pentaquine is administered alone and concurrently with other drugs

CONCURRENT DRUGS	NUMBER OF SUBJECTS IN GROUP	MEAN CONCENTRATION OF PENTAQUINE (SN-13,276) IN THE PLASMA DURING THERAPY*	MEAN METHEMOGLOBIN CONCENTRATION DURING LAST FIVE DAYS OF THERAPY**
		gamma of base per liter	*per cent of total hemoglobin*
Pentaquine	10	42	4.5
Quinine	82	59	4.3
Paludrine	5	270	4.5
Chloroquine	5	52	6.8
Quinacrine	5	130	12.5
SN-11,437	5	8	4.5
Sulfadiazine and Quinine	4	24	6.5

* Brodie et al. (1947).

** When determined by Wendell's modification of the Evelyn and Malloy (1938) method, the mean methemoglobin of 187 normal individuals was 1.8 per cent of the total hemoglobin (standard deviation 1.0). This value has not been subtracted from the mean values given above.

Several 8-aminoquinolines (Alving, et al.), including pamaquin (plasmochin) in high dosages (Zubrod, et al., 1947), will occasionally cause granulocytopenia or agranulocytosis.[5] It is also well established that agranulocytosis may at times result from sulfonamide therapy. The occurrence of three instances of granulocytopenia or agranulocytosis, however, in nine patients receiving an 8-aminoquinoline (pentaquine) concurrently with a sulfonamide (SN-11,437 or sulfadiazine) emphasizes the danger of sulfonamide therapy for intercurrent infections during treatment of malaria with pentaquine, at least when high dosages of the latter drug are used, and suggests caution in the use of sulfonamides during pamaquin therapy.

[5] Granulocytopenia has not been observed by us in 211 white patients to whom pentaquine has been administered in doses of 180 mgm. of the base or less. However, Fries et al. have recently reported severe granulocytopenia in one of seventeen hypertensive patients receiving 120 to 240 mgm. of the base per day.

SUMMARY

1. Concurrent administration of quinacrine with pentaquine increased some of the toxic manifestations of pentaquine. A marked increase in severity of methemoglobinemia was noted. Mild hemolytic anemia was observed in one of five white patients with malaria, receiving 80 mgm. of pentaquine monophosphate (60 mgm. base) and quinacrine concurrently.

2. Of five white patients with malaria, receiving 80 mgm. of pentaquine monophosphate and SN-11,437 (a drug chemically related to sulfadiazine) concurrently, one developed granulocytopenia and one developed agranulocytosis. Agranulocytosis was observed in one of four normal white volunteers receiving pentaquine, quinine, and sulfadiazine concurrently. These observations emphasize the potential danger of sulfadiazine therapy during administration of pentaquine.

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STUDIES ON IMPORTED MALARIAS

8. Infectivity to *Anopheles quadrimaculatus* of Asymptomatic *Plasmodium vivax* Parasitemias[1]

DON E. EYLES, MARTIN D. YOUNG and R. W. BURGESS[2]

Division of Tropical Diseases, Office of Malaria Investigations, Colombia, S. C.

(Received for publication 5 May 1947)

Previous investigations have demonstrated that asymptomatic parasitemias often exist in persons infected with *Plasmodium vivax* returning from tropical military service. Since persons with parasites in the peripheral blood but without symptoms would be more likely to become exposed to mosquitoes than patients in bed or in hospitals, the likelihood of mosquito infection becomes epidemiologically important. The infectivity of such asymptomatic malarias to *Anopheles quadrimaculatus* is the basis of this report.

An accompanying paper in this series (Eyles and Young, 1948) outlines the types of asymptomatic parasitemia encountered in a study of a large group of Army malaria patients. Referring to this paper, asymptomatic parasitemias may occur as follows:

1. Just preceding relapse, parasites may be present in the blood for a period varying from one to several days and are defined as "preclinical parasitemias."
2. During the interval between clinical episodes patients may have parasites without symptoms; these parasitemias are usually transient and low in level and are defined as "interval parasitemias."
3. Following the last clinical expression of the disease, asymptomatic parasitemias may occur as a final phase of the disease in man; these episodes are defined as "terminal parasitemias."

The same patient only rarely had asymptomatic parasitemias of all three types.

METHODS

Insectary reared *Anopheles quadrimaculatus* mosquitoes varying from 30 to 100 females per lot were fed on volunteer Army patients with asymptomatic parasitemias using the method described by Burgess and Young (1944). These mosquitoes were dissected after sufficient time (about 10 days) had elapsed to allow easy counting of the oocysts, but before sporozoites had reached the salivary glands. If the infections were low in intensity or absent the entire lot was dissected at this time. If the infection was heavier a few mosquitoes were incubated longer to demonstrate that the infection was completed to the glands.

[1] Contribution from the Imported Malaria Studies program of Malaria Investigations, National Institute of Health, and the Office of Malaria Control in War Areas, United States Public Health Service, Columbia, S. C.

[2] Moore General Hospital and Fort Jackson (S. C.) Regional Hospital made the relapsing cases available. To these and especially to Moore General and the South Carolina State Hospital, which also furnished laboratory quarters, we express appreciation. We are also indebted to the Office of The Surgeon General, United States Army, whose active interest made the program possible.

The group of random feedings on symptomatic parasitemias used for comparison was secured in an essentially similar manner.

Parasitological follow-up of the patients was outlined in an accompanying paper (Eyles and Young, 1948). Gametocyte counts were restricted to males because of the fact that most quantitations were made from thick blood films in which female gametocytes are difficult to recognize.

OBSERVATIONS

Infectivity of asymptomatic parasitemias. A total of 118 lots, comprising 2,059 mosquitoes dissected, were fed on 35 patients with asymptomatic parasitemias. Patients were originally chosen at random and repeated feedings on the same patient were made whenever practicable. One patient was fed upon 57 times; one patient, twelve times; one, six times; one, four times; one, three times; six, twice. The other 24 patients were fed upon a single time.

Of the total, 28.0 per cent of the lots were infected and 11.6 per cent of the mosquitoes in all lots were infected. Of the individual patients, about 34 per cent at one time or another infected mosquitoes while asymptomatic.

Feedings were made on patients from both the Mediterranean and Pacific theaters; the results being similar, these are not discussed separately.

Table 1 summarizes these feedings by the type of asymptomatic parasitemia present. It will be noted that infections resulted from each type in some lots, which would indicate that malaria patients are potential infectors whenever parasites are present.

Eight of 21 lots (38.1 per cent) fed on patients with preclinical asymptomatic parasitemia were infected; however, due to the relatively small number of lots, the error of this sample is large (S.E. = 10.6). A little over one-fifth of all mosquitoes applied became infected and the mean number of cysts per infected gut was relatively high (27.4).

Patients with interval asymptomatic parasitemia were fed upon only three times. Two lots (both fed on the same patient) were infected. The sample is admittedly small but it demonstrates that these interval asymptomatic parasitemias are sometimes infective. The percentage of all mosquitoes infected was high (45.1), but the number of cysts per infected gut was relatively low (6.4).

The largest group of feedings was made on patients with terminal asymptomatic parasitemia. About one-quarter of 94 lots fed on such patients became infected. Infections were generally of low intensity (7.5 per cent of 1,555 mosquitoes infected with a mean of 9.4 cysts per infected gut).

Serial feedings were made on several patients with terminal asymptomatic parasitemias. In each case mosquito infection rates were highest early during the course of the parasitemia when parasite counts were also highest. Figure 1, based on 51 daily feedings starting on the first day of the asymptomatic parasitemia upon a single patient, illustrates this fact typically. It is shown that mosquitoes were infected 13 times during the first 42 days but not at all thereafter. There were six additional feedings on this patient between the 85th and 134th day of asymptomatic parasitemia; no infections in mosquitoes resulted.

Except for the small group of feedings on patients with interval asymptomatic parasitemia, the largest proportion of infected lots was in the group fed on patients

TABLE 1

Mosquito infections resulting from feedings on patients with asymptomatic Plasmodium vivax *parasitemias*

	TYPE OF PARASITEMIA			
	Preclinical	Interval	Terminal	All
Number of patients	17	2	16	35
Mosquito lots fed	21	3	94	118
Mosquito lots infected	8	2	23	33
Per cent lots infected	38 ± 11	66 ± 28	25 ± 4	28 ± 4
Number of mosquitoes dissected	433	71	1,555	2,059
Per cent mosquitoes infected	21.2	45.1	7.5	11.6
Mean number of cysts per infected gut	27.4	6.4	9.4	14.8

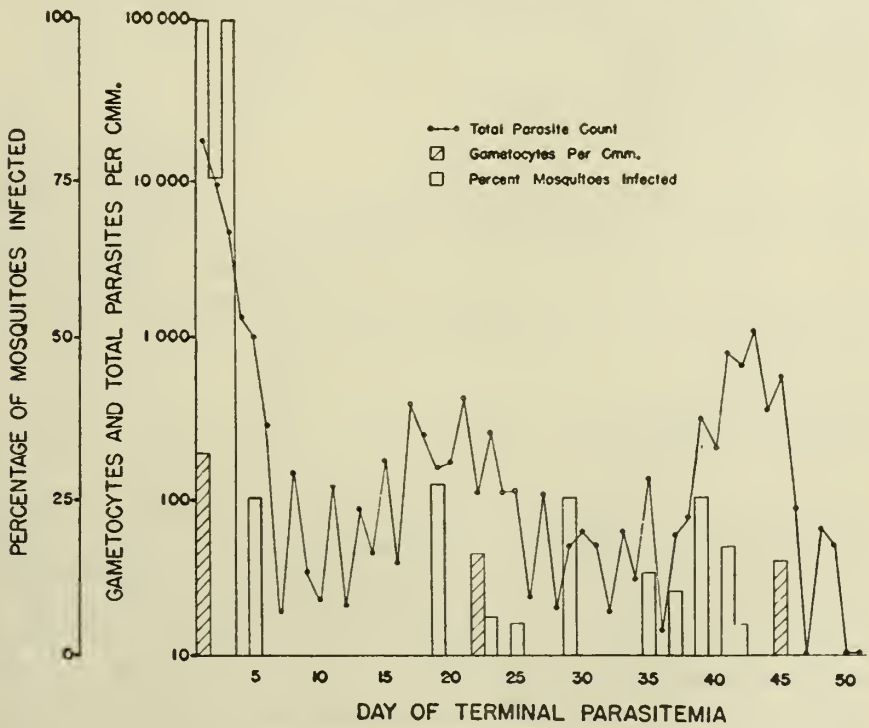

FIG. 1. *P. vivax* infections in *A. quadrimaculatus* resulting from 51 consecutive daily feedings upon a single patient. Feedings were started on the first day of a terminal asymptomatic parasitemia. Not shown in the figure are six additional feedings between the 85th and 134th day of asymptomatic parasitemia from which no mosquitoes were infected.

with preclinical parasitemia. The difference between the proportion infected by the latter (38.1 per cent) and the proportion infected by the terminal asymptomatic para-

sitemias (24.5 per cent) would occur by chance one in four times, all factors being equal. The fact that more cysts per infected gut and a larger proportion of infected mosquitoes were found in the individual lots fed on patients with preclinical parasitemia may indicate a greater degree of significance than was disclosed by the statistical method used.

Relative infectivity of asymptomatic and symptomatic parasitemias. Table 2 compares the entire group of 118 feedings on asymptomatic patients with a series of 114 random feedings on clinical cases. The lots fed on the clinical cases were more highly infected, not only as to the percentage of lots infected (49.1 per cent against 28.0 per

TABLE 2

Comparison of mosquito feedings on clinical cases versus feedings on patients with asymptomatic Plasmodium vivax *parasitemias*

	CLINICAL CASES	ASYMPTOMATIC CASES
Mosquito lots fed	114	118
Mosquito lots infected	56	33
Per cent lots infected	49.1 ± 4.7	28.0 ± 4.1
Number mosquitoes dissected	3,853	2,059
Per cent mosquitoes infected	24.9	11.6

TABLE 3

Comparison of mosquito feedings during clinical episodes and during terminal asymptomatic parasitemia on the same patient. Plasmodium vivax

	CLINICAL PERIOD	ASYMPTOMATIC PERIOD
Mosquito lots fed	3	57
Mosquito lots infected	3	14
Per cent lots infected	All	24.6
Number mosquitoes dissected	68	782
Per cent mosquitoes infected	87	6.1

cent), but also as to percentage of mosquitoes infected. Mean number of cysts per infected gut was not recorded for all of the symptomatic lots but when noted were higher than in the asymptomatic group. Statistical comparison based on the standard errors of the percentages of lots infected demonstrates a high degree of significance to the difference ($p = .0018$).[3] It should be borne in mind that 57 of the asymptomatic feedings were made on a single patient. Three additional feedings were made on this same patient when he was ill. Table 3 compares these feedings; despite the

[3] A more nearly random statistical comparison was made between the symptomatic feedings as given here and the first feeding on each of the 35 asymptomatic volunteers. Of the asymptomatic cases, 34.3 ± 8.0 per cent were infected (S.E. 8.024) against 29.1 ± 4.7 per cent of the symptomatic. Probability in this case is 0.112 which is above the usual level of significance, but the difference appears more significant when it is considered that the individual symptomatic lots had over twice as many mosquitoes infected.

small number of feedings while this patient was ill, the lower infection values when the patient was asymptomatic are highly significant (p = .0002).

Parasite and gametocyte density as related to infectivity. Table 4 compares the mean total parasite and male gametocyte densities of the three types of asymptomatic parasitemia with the symptomatic at time of feeding. This tabulation also indicates the proportion of patients with patent gametocytes and repeats the proportion of mosquito lots infected. Infection in the mosquitoes is seen to correspond well both with the percentage of patients with male gametocytes and the mean male gametocyte density.

In the case of the preclinical asymptomatic parasitemia we believe the group of patients upon whom mosquitoes were fed to be representative of the larger number of patients whose cases were followed parasitologically. Eyles and Young (1948) state that during the preclinical phase parasitemias rise steadily from scarcely detectable

TABLE 4

Parasite levels at time of feeding and proportion of smears with male gametocytes compared with proportion of mosquito lots infected. Plasmodium vivax

TYPE OF PARASITEMIA	MEAN COUNT PER CMM.		PER CENT SMEARS WITH GAMETOCYTES	PER CENT MOSQUITO LOTS INFECTED
	Total parasites	Male gametocytes		
Preclinical asymptomatic	1,763	25	19.0	38.1
Interval asymptomatic	163	78	66.7	66.7
Terminal asymptomatic	606	4	8.5	24.5
All asymptomatic	800	10	11.9	28.0
Symptomatic*	6,577	46	50.6	49.1

* These data are from 77 of the 114 symptomatic feedings; only those cases in which both total count and male gametocyte count were known were tabulated.

levels to levels sufficiently high to provoke symptom response (mean fever threshold count at relapse observed by Eyles and Young to be 6300 per cmm.). The mean total parasite level observed in our group of patients would be near that which would be expected in a random sample. The mean male gametocyte level observed in the group of patients upon whom mosquitoes were fed is also such as might be expected (gametocyte count at relapse 46 per cmm.).

We do not believe, however, that the group of three patients with interval asymptomatic parasitemias upon whom mosquitoes were fed to be representative. Table 5 presents data based on the study of 24 patients with this type parasitemia and it can be seen that the incidence of gametocytes and the ratio of male gametocytes to total parasites is much lower in the large group than in the group upon whom mosquitoes were fed. Consequently, the proportion of lots infected on these patients is almost certainly misleadingly large. Eyles and Young (1948) state that total parasite level in this type of asymptomatic parasitemia is low; lower, in fact, than in the other types of asymptomatic parasitemias. This fact together with the fact that no great dissimilarity in the ratio of gametocytes to total parasites exists in this group of patients

when symptomatic and when asymptomatic is further evidence of the non-representative nature of our group of volunteers.[4]

The group of patients with terminal asymptomatic parasitemias upon whom mosquitoes were fed is apparently quite representative of the larger group studied parasitologically. Table 5 analyzes data on 76 patients and the proportion of smears with gametocytes in this group is very similar to the proportion in the smears made coincident with feeding (6.3 per cent as compared with 8.5 per cent).

Eyles and Young (1948) showed that parasite levels during the terminal asymptomatic parasitemia are seldom higher than one-quarter to one-half of those noted during the clinical attack, the level gradually decreasing after onset of parasitemia. The fact that in the same patients only about one-third as many smears with gametocytes evident were found when terminally asymptomatic as when clinically ill is undoubtedly due to the lower parasite levels as the ratio of male gametocytes to total parasites was similar during parasitemias with and without symptoms (table 5).

TABLE 5

The incidence of male gametocytes in interval and terminal asymptomatic parasitemias compared with parasitemias accompanied by symptoms in the same patients. Plasmodium vivax

	CLINICAL PARASITEMIA	INTERVAL ASYMPTOMATIC PARASITEMIA	CLINICAL PARASITEMIA	TERMINAL ASYMPTOMATIC PARASITEMIA
Number of patients	24	24	76	76
Total number of smears	142	118	326	907
Per cent smears with gametocytes	19.0	6.8	21.2	6.3
Ratio of number of gametocytes to total parasites	0.0048	0.0107	0.0083	0.0081

Tables 6 and 7 compare infection rates in the mosquitoes in the symptomatic group with those in the asymptomatic group at different male gametocyte and total parasite levels. It will be noted that in both the asymptomatic and the symptomatic groups at least a fifth of the lots were infected when no male gametocytes were discerned (less than 10 per cmm.). In spite of apparent dissimilar infection rates, in no case at the various levels of male gametocyte and total parasite density are the differences between the corresponding proportions of lots of mosquitoes infected on asymptomatic and symptomatic parasitemias statistically significant. Simply stated, the evidence indicates that similar parasite or gametocyte numbers are equally effective in infecting mosquitoes in both the asymptomatic and symptomatic patients.

From the preceding tables (4, 6, and 7) it would appear that infection in the mosquito is positively correlated both with total parasite count and male gametocyte count. In order to determine which count would be the more reliable criterion to use in estimating potential infectivity of asymptomatic patients the relationship between intensity of infection in the mosquitoes and parasite or male gametocyte density was measured by means of the Pearsonian coefficient of correlation (r) for the entire group

[4] Such dissimilarity as exists in the ratios is due to the inclusion of a single high gametocyte count in one asymptomatic patient, this patient being the one upon whom two lots of mosquitoes were fed.

of asymptomatic feedings. For the mosquitoes an index of intensity of infection was expressed in such a manner as to take into regard both the percentage of mosquitoes infected in each lot and the number of cysts per infected gut (infection index = per cent mosquitoes infected × mean number of cysts per infected gut).

Coefficient of correlation (r) between male gametocyte density and infection index was found to be about +0.7. By means of the Z transformation it was found that this value was not significantly different from a hypothetical r value of 1.0 (p = 0.22).

TABLE 6

Mosquito infection rate (percentage of lots infected) in relation to male gametocyte count. Plasmodium vivax

MALE GAMETOCYTES PER CMM.	PER CENT OF LOTS INFECTED			
	Asymptomatic group		Symptomatic group	
	Lots fed	Per cent infected	Lots fed	Per cent infected
Less than 10*	104	20.0	50	22.0
10–20	8	75.0	14	42.9
Over 20	6	100.0	50	78.0

* No gametocytes found in 0.1 cmm. of blood.

TABLE 7

Mosquito infection rate (per cent of lots infected) in relation to total parasite count. Plasmodium vivax

TOTAL PARASITE COUNT PER CMM.	PER CENT OF LOTS INFECTED			
	Asymptomatic group		Symptomatic group	
	Lots fed	Per cent infected	Lots fed	Per cent infected
1–50	46	6.5	3	0.0
51–250	31	19.4	1	0.0
251–1250	26	34.2	9	22.2
1251–6250	12	75.0	33	36.4
6251 and over	3	100.0	27	66.7

Coefficient similarly calculated for total parasite density and infection index for the same feedings was about +0.5. This value was compared with the value of +0.7 found above by means of the Z transformation and the two were significantly different (p = .0005).

From the above it would appear that, despite the fact that infection resulted in over one-fifth of the instances in which mosquitoes were fed on patients in which gametocytes were sub-patent, the best estimate of the potential infectivity of a patient can be made on the basis of the male gametocyte count. It should be pointed out that correlations on the basis of either total or female gametocyte count were not attempted.

In those cases in which no gametocytes were observed, infection in the mosquitoes

was positively correlated with the total parasite count ($r = + 0.3$), but not to a degree sufficient to estimate accurately the likelihood of infection.

DISCUSSION

As was stated in introducing this paper, persons with asymptomatic malaria parasitemias are more likely to expose themselves to mosquitoes than ill patients. It has been recognized for some time that returning servicemen are at times asymptomatic carriers of *Plasmodium vivax*, but to this time neither the incidence of the asymptomatic parasitemias nor the degree to which they can infect mosquitoes has been adequately estimated.

In an accompanying paper Eyles and Young (1948) have demonstrated that a significantly large proportion of servicemen with relapsing malaria will have these asymptomatic periods. It was shown that two-thirds of a large group of individuals had detectable numbers of parasites for several days prior to the onset of relapse symptoms; twelve per cent showed small numbers of parasites during the interval between clinical episodes; and in 25 per cent of the individuals observed the termination of the disease came as an asymptomatic parasitemia which often endured for long periods of time. It was shown also that asymptomatic parasitemias prevail several times as long as symptomatic parasitemias.

Since a large number of ex-servicemen will carry on their normal activities while still harboring parasites, the assessment of the hazard they will constitute to the community is of vital importance.

Our evidence shows that, qualitatively, the malaria patient can infect mosquitoes throughout the duration of the disease, whenever parasites are present in the peripheral blood. Our evidence shows, furthermore, that the degree to which mosquitoes are infected is low during asymptomatic periods, being proportional to the male gametocyte count and the total parasite count which are also low as compared with the symptomatic periods.

These findings, based upon 118 feedings on asymptomatic patients, are not in accord with those of Watson (1945a, 1945b) who found higher infection rates in 7 lots of mosquitoes fed on asymptomatic patients than in a larger number of lots fed on patients with symptoms. As was pointed out by Christianson *et al.* (1946), comparison with Watson's work is difficult because of the fact that Watson did not state the percentage of patients who actually infected mosquitoes.

We found a lower incidence of gametocytes in smears made upon asymptomatic patients than in smears made on ill patients. Similar ratios of gametocytes to total parasites were found in asymptomatic and symptomatic parasitemias, the lower incidence of gametocytes in asymptomatic smears probably being due to the much lower parasite levels. The higher incidence of gametocytes in fever-free patients reported by Christianson *et al.* (1946) is not statistically significant for the number of cases and smears they studied.

Taking all factors into consideration, it would appear that the low infectivity to mosquitoes of asymptomatic malaria is offset by the frequent occurrence and relatively long prevalence of these symptomless parasitemias. In areas very favorable to mosquito transmission, the asymptomatic patient would be a definite hazard.

SUMMARY AND CONCLUSIONS

1. A total of 118 lots of *Anopheles quadrimaculatus* comprising 2,059 mosquitoes dissected were fed on 35 asymptomatic carriers of foreign *Plasmodium vivax*. The results demonstrated that malaria patients are potentially infective to mosquitoes whenever parasites are present.

2. Infection was significantly lower in asymptomatic cases than in a random group of 114 feedings comprising 3,853 mosquitoes dissected fed on patients with symptoms, not only as to percentage of lots infected but also as to percentage of mosquitoes infected.

3. Infection in the mosquitoes was positively correlated with the number of male gametocytes present, but about one-fifth of the lots, both of the asymptomatic and symptomatic group, were infected when fewer than 10 male gametocytes per cmm. were present.

4. Infection in the mosquitoes fed on asymptomatic and symptomatic carriers varied directly in intensity with the total parasite count, the lower parasite counts of the asymptomatic cases resulting in lower infection values.

5. Male gametocytes were observed to have a lower incidence in smears taken on asymptomatic patients, the lower incidence being due to lower levels of parasitemia.

6. No evidence was found to indicate an increased production of gametocytes during asymptomatic periods. Similar ratios of male gametocytes to total parasites held for asymptomatic and symptomatic parasitemias.

7. On the basis of these results, it is concluded that patients showing asymptomatic parasitemias of foreign *vivax* malarias can infect mosquitoes but at a lower rate than those with symptomatic parasitemias. However, as the asymptomatic patient is more likely to be exposed to malaria vectors than the ill patient, the overall hazard of the asymptomatic malaria carrier may be as great or greater than that of the one clinically ill.

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STUDIES ON IMPORTED MALARIAS

9. The Comparative Susceptibility of *Anopheles quadrimaculatus* and *Anopheles maculipennis freeborni* to Foreign *Vivax* Malaria[1,2]

MARTIN D. YOUNG and ROBERT W. BURGESS

Office of Malaria Investigations, Division of Tropical Diseases, National Institute of Health, Columbia, S. C.

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Anopheles quadrimaculatus and *A. m. freeborni* are considered to be the principal malaria vectors in the United States of America. Earlier reports (Moore *et al.*, 1945; Young *et al.*, 1946) have shown that these species of mosquitoes were susceptible to and transmitted foreign *Plasmodium vivax* malaria relapsing in returned military personnel.

The object of this report is to compare the susceptibility of these species of mosquitoes to foreign *vivax* malaria.

METHODS

The procedures followed have been described earlier (Burgess and Young, 1944; Moore *et al.*, 1945). In general, they consisted in feeding mosquitoes (preferably 100 or more of each species) simultaneously upon a patient, incubating the mosquitoes in a temperature-controlled insectary (74°–78°F.), and dissecting the mosquitoes at intervals to determine the infection rates. To compare the number of oocysts, counts were made from the 7th day of incubation until sporozoites appeared in the salivary glands. Oocyst counts made before or after this period were not used for comparison.

The Q-1 strain of *A. quadrimaculatus* and the F-1 strain of *A. m. freeborni* were used. The mosquitoes were applied to soldiers with clinical relapsing foreign *vivax* malaria or to neurosyphilitic patients with primary attacks of foreign *vivax* malaria which had been induced either by transfer of infected blood or the bites of infected mosquitoes.

The malarias had originated presumably in the following areas: Solomon Islands, New Hebrides Islands, New Guinea, Tunisia, Liberia, Trinidad, and the China-Burma-India theater.

OBSERVATIONS

About 50 feedings were made. Only those (24) in which either one or both species of mosquitoes became infected are shown in the tabulations (table 1).

[1] Contribution from the Imported Malaria Studies program of the Division of Tropical Diseases, National Institute of Health, and the Office of Malaria Control in War Areas, United States Public Health Service, Columbia, S. C.

[2] The Fort Jackson (S. C.) Regional Hospital made relapsing malaria cases available and the active interest of the Office of the Surgeon General, U. S. Army, made the program possible. The South Carolina State Hospital furnished laboratory quarters. To these we express our appreciation.

Measured by the percentage of mosquitoes which were infected, *A. m. freeborni* was significantly more susceptible than *A. quadrimaculatus* to the malaria from the standpoints of (a) relapsing infections in the soldiers (2.4 S.E.), (b) the primary infections in the neurosyphilitic patients (2.7 S.E.), or (c) the total of both (3.6 S.E.). In 4 instances where the *A. quadrimaculatus* were not infected, the *A. m. freeborni* showed the following infection percentages: 18, 7, 3, and 5. In 20 of the 24 feedings, a higher percentage of *A. m. freeborni* than *A. quadrimaculatus* was infected.

To further compare the susceptibility the oocysts on the infected mosquito guts were counted. The *A. m. freeborni* rather consistently had more oocysts per infected gut than did the *A. quadrimaculatus*, and the mean was higher in the former (18.7 as compared to 15.2).

TABLE 1

Comparison of resulting infections when Anopheles quadrimaculatus *and* A. m. freeborni *were fed simultaneously upon foreign* vivax *malaria*

The malarias were either relapsing infections in returned soldiers or primary infections induced in white neurosyphilitic patients.

PATIENT SOURCE	NUMBER OF FEEDINGS	*A. quadrimaculatus*				*A. m. freeborni*				TOTAL MOSQUITOES	
		Mosquitoes		Infected guts		Mosquitoes		Infected guts			
		Dissected	Per cent infected*	Number of guts	Average number of oocysts	Dissected	Per cent infected*	Number of guts	Average number of oocysts	Per cent infected*	S.E.†
Soldiers	18	735	40.1	132	18.6	625	46.6	155	22.2	43.1	2.4
Neurosyphilitics	6	186	60.2	64	8.2	205	71.2	69	10.9	66.0	2.7
Total	24	921	44.2	196	15.2	830	52.7	224	18.7	48.2	3.6

* An infected mosquito is one showing oocysts, sporozoites, or both.

† Standard error between the means of *A. quadrimaculatus* and *A. m. freeborni*.

DISCUSSION

It had been noticed in previous work that *A. m. freeborni* showed a higher infection rate, 42.4 per cent (Moore *et al.*, 1945), than *A. quadrimaculatus*, 30.8 per cent (Young *et al.*, 1946), when fed upon relapsing foreign *vivax* malarias. However, as these species of mosquitoes had been tested in separate laboratories and with different patients, it was not certain that the tests were comparable. Consequently, the present work was performed where the 2 mosquito species were tested under identical conditions.

Considering the proportion of mosquitoes becoming infected, and the number of oocysts per infected gut, it appears that *A. m. freeborni* is more susceptible than *A. quadrimaculatus* to foreign *vivax* malaria.

Previously, 4 anophelines of the southern United States, viz., *A. punctipennis*, *A. quadrimaculatus*, *A. pseudopunctipennis pseudopunctipennis*, and *A. albimanus*, were compared as to their relative susceptibility to foreign *vivax* malaria (Young *et al.*,

1946). *A. quadrimaculatus* was used as the standard for comparison in the above tests as well as in the tests with *A. m. freeborni*; therefore, it is possible to assign relative values. Giving *A. quadrimaculatus* a value of 100, the relative values of the other species are shown in table 2. Theoretically one might draw up an evaluation showing what percentage of each species might be expected to become infected in the same situation where the most susceptible species showed a 100 per cent infectivity (table 2).

Also shown in table 2 are the standard errors between the means of the different species tested and *A. quadrimaculatus*.

A. m. freeborni appears to be significantly more susceptible than *A. quadrimaculatus*, while *A. p. pseudopunctipennis* and *A. albimanus* are significantly less susceptible. *A punctipennis* appears to have about the same susceptibility as *A. quadrimaculatus*,

TABLE 2

Comparison of susceptibility to foreign vivax *malaria of 4 anopheline species to* A. quadrimaculatus *showing the standard errors between the means, the relative numerical evaluation, and the theoretical percentage of infection where the most susceptible species would show a 100 per cent infectivity*

	STANDARD ERROR*	RELATIVE EVALUATION	THEORETICAL PERCENTAGE EVALUATION
A. m. freeborni	3.6	119	100
A. punctipennis	0.1	102	86
A. quadrimaculatus		100	84
A. p. pseudopuntipennis	5.1	41	35
A. albimanus	20.7	2	2

* Standard error between means of particular species and *A. quadrimaculatus*. The means for *A. punctipennis*, *A. p. pseudopunctipennis*, and *A. albimanus* were given in a previous report (Young *et al.*, 1946).

but the number of mosquitoes involved were small, viz., 46 and 83 respectively, so that the test might not be adequate.

SUMMARY AND CONCLUSIONS

Anopheles quadrimaculatus and *A. m. freeborni* were applied simultaneously to patients with either naturally acquired or induced foreign *Plasmodium vivax* malaria. The malarias had originated from widely separated areas.

A. m. freeborni showed more oocysts per infected gut and a significantly higher percentage of mosquitoes infected than *A. quadrimaculatus*.

It is concluded that *A. m. freeborni* is more susceptible to these foreign malarias than *A. qudrimaculatus*.

The theoretical relative evaluation of susceptibility to foreign *vivax* malaria of 5 species of anophelines, using the most susceptible as 100, would be: *A. m. freeborni*, 100; *A. punctipennis*, 86; *A. quadrimaculatus*, 84; *A. p. pseudopunctipennis*, 35; and *A. albimanus*, 2. The differences between the various species and *A. quadrimaculatus* were significant for except *A. punctipennis*.

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OBSERVATIONS ON THE DURATION OF TOXICITY OF DDT TO *ANOPHELES QUADRIMACULATUS* SAY UNDER FIELD CONDITIONS

ALBERT A. WEATHERSBEE, F. T. ARNOLD, JR. AND JULIAN P. HOPKINS

Malaria Control Division, South Carolina State Board of Health, Columbia, S. C.

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Under the auspices of the Malaria Control Division of the South Carolina State Board of Health, five experiments were conducted to determine the duration of toxicity of varying concentrations of DDT to *Anopheles quadrimaculatus* Say under field conditions. From a stock solution of 35 per cent technical grade DDT in xylene, with triton X-100 as an emulsifying agent, watery emulsions of 1, 2, 3, 4, and 5 per cent were prepared. Five stations were selected in Calhoun County, South Carolina, along the upper part of the Santee Reservoir. Ten mosquito counts on ten separate days were made in each of them before the experiments were started. The mosquito population within this area is comprised almost entirely of *A. quadrimaculatus*.

Stables were used in experiments number 1, 2, and 4; a farm house in number 3; and two dog houses in number 5. These places showed average counts ranging from 30 to 92 anopheline mosquitoes during ten preliminary observations.

On May 9, 1946, in experiment No. 1, a one per cent DDT emulsion was sprayed on the walls and ceiling of stable number 1 in such manner as to provide a theoretical deposit of 40 mgm. DDT per square foot of surface area. The adjoining stable was not sprayed. Observations were made in search of mosquitoes twice each week after spraying, and mosquitoes encountered were collected and identified.

After twenty-seven days, on June 6, five *A. quadrimaculatus* were found in the sprayed stable. Abnormal activity and apparently weakened condition of these mosquitoes indicated that they had been exposed to DDT. They were observed for an hour and a half, after which they flew to unsprayed areas. *A. quadrimaculatus* were present during each succeeding observation, but were fewer than in the unsprayed stable serving as a control.

After fifty-three days, the number of *A. quadrimaculatus* found in the sprayed stable was approximately the same as in the unsprayed stable. Observations were continued to ninety days.

Before spraying, the average of ten counts was 41 in the stable to be sprayed and 43 in the other one. After spraying the one stable, the average count of mosquitoes in the adjoining unsprayed stable dropped to 6.5. Even after ninety days, the counts in the stables had not approached the pre-spraying densities.

For experiment No. 2, the same procedure was used except a 2 per cent emulsion was sprayed in one stable so as to provide a theoretical deposit of 80 mgm. DDT per square foot, and the adjoining stable was not sprayed. This experiment was started the same days as experiment No. 1.

After thirty-five days, four *A. quadrimaculatus* were found in the sprayed stable. Their actions were not normal, and they were considered to be sick, but flew to unsprayed areas before dying. Results of experiment No. 2 followed the same general pattern as those of experiment No. 1. After spraying, the count of mosquitoes dropped from 35 to zero in the sprayed stable, and was markedly reduced in the adjoining unsprayed stable.

The number of mosquitoes in the sprayed stable increased after the thirty-fifth day after spraying until on the seventy-seventh day, it was equal to that in the unsprayed stable; although neither the sprayed or the unsprayed stable supported numbers equal to those present before spraying. At this time, numerous flies and other insects had returned to the wall surfaces.

For experiment No. 3, a farm house was selected. The house was built about six feet above the ground. The space under the house was generally cool and slightly damp, and provided an excellent resting place for *A. quadrimaculatus*. The underside of the house was divided into two sections. On May 15, one section was sprayed with a 3 per cent DDT emulsion, so as to provide a residual deposit of 120 mgm. DDT per square foot of surface area. The adjoining section was not sprayed. Before spraying, the mosquito count for each section exceeded 75 *A. quadrimaculatus*.

Immediately after spraying, the average count in the unsprayed section dropped to 16.5. However, during heavy rainfalls, the numbers present exceeded this number. In the sprayed section, the count was zero until the sixty-third day when seven *A. quadrimaculatus* and one *Anopheles crucians* Wied. were found. Continual checking for ninety-one days showed that the mosquito count in the sprayed section was still lower than that in the unsprayed section. During this period, the average number of mosquitoes found in the sprayed section never exceeded 14, whereas the average number found per observation in the unsprayed section reached 88 in the seventh week. After sixty-three days, the ratio was two in the unsprayed section to one in the sprayed section.

For experiment No. 4, two adjoining stables were used. One was well sprayed on May 16 with 4 per cent DDT emulsion providing a residue of 160 mgm. per sq. foot of surface area. The other was not sprayed. In observations made twice a week thereafter, *A. quadrimaculatus* made its first appearance in the sprayed stable 102 days after it had been sprayed.

The mosquito count for the stable that was not sprayed dropped from 34 to an average of 9.5 in the second week after spraying.

In experiment No. 5, two adjoining dog houses were used. The mosquito count was high in each of them. One house was sprayed on May 17 with 5 per cent DDT emulsion providing a residue of 200 mgm. per sq. foot. The other house was not sprayed.

During 135 days of continual checking, no mosquitoes were found in the sprayed dog house. At this time, observations were discontinued. Mosquito counts per observation in the unsprayed dog house dropped from 92 before spraying to 12 after spraying. Data pertaining to inspection records and mosquito counts during the experiments are shown in table 1, and presented graphically in charts II, III, IV, V, and VI.

Chart I shows the time interval after spraying with the different concentrations of DDT until the first appearance of *A. quadrimaculatus* in the sprayed shelters. It is apparent that as the concentration is increased, the duration of time until *A. quadrimaculatus* appears also increases. The greatest increase in time duration is that accompanying the increase in concentration from 3 per cent to 4 per cent. The toxicity of the 2 per cent concentration as determined by the presence or absence of *A. quadrimaculatus* persisted only a few days longer than that of the 1 per cent and 5 per cent somewhat longer than 4 per cent. However, these differences are not so great as that between 3 per cent and 4 per cent concentrations.

TABLE 1

Average number of resting An. quadrimaculatus *on sprayed surfaces correlated with length of time after spraying*

WEEKS AFTER SPRAYING	1 PER CENT DDT		2 PER CENT DDT		3 PER CENT DDT		4 PER CENT DDT		5 PER CENT DDT	
	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control	Sprayed	Control
	Average station counts before spraying									
	41	43	35	32	78	76	30	34	89	92
	Average station counts after spraying									
1	0.0	6.5	0.0	1.0	0.0	16.5	0.0	0.0	0.0	12.0
2	0.0	13.0	0.0	0.0	0.0	30.0	0.0	9.5	0.0	13.0
3	0.0	21.0	0.0	9.0	0.0	52.5	0.0	11.0	0.0	9.0
4	2.5	21.5	0.0	20.0	0.0	34.0	0.0	5.0	0.0	47.0
5	5.0	10.5	2.0	9.0	0.0	64.0	0.0	10.5	0.0	52.0
6	6.0	9.5	2.5	7.0	0.0	42.5	0.0	10.0	0.0	39.0
7	4.0	7.0	2.0	5.0	0.0	88.0	0.0	7.0	0.0	32.0
8	7.5	10.0	3.5	8.0	0.0	62.0	0.0	16.5	0.0	45.5
9	9.0	10.5	6.5	11.0	4.0	50.5	0.0	11.0	0.0	39.0
10	8.0	8.5	5.5	8.0	2.5	26.0	0.0	14.0	0.0	42.5
11	10.0	7.5	12.0	12.5	12.0	31.0	0.0	18.0	0.0	40.5
12	15.0	13.0	11.0	9.0	13.5	26.1	0.0	11.5	0.0	44.0
13							0.0	14.0	0.0	50.5
14							0.0	12.5	0.0	36.0
15							1.5	10.5	0.0	32.5
16							1.0	9.0	0.0	36.5

CAGE TESTS

Associated with the observed loss of toxicity of DDT in the concentrations used, as indicated by the failure to maintain the sprayed areas mosquito-free, it was desirable to measure the extent of loss of toxicity of each of the sprayed surfaces under consideration. For this purpose, five female *A. quadrimaculatus* were placed in each of 30 cages. Three of these cages were attached to the sprayed surfaces of each concentration of DDT with the netting removed on the contact side so that the mosquitoes were exposed directly to the sprayed surfaces. Exposure in this manner was continued for 15 minutes in each case. At the same time, three companion cages were carried into the structures and left on a chair, table, or floor, while the exposure cages were being exposed to the sprayed surfaces. Mosquitoes in these companion

cages served as controls against the exposed mosquitoes. After exposure was accomplished, the exposure cages and the control cages were transported to the laboratory where they were kept at room temperature with moist pads on the netting. At varying intervals, the caged mosquitoes were observed, and dead mosquitoes were counted. The results of these tests are shown in accompanying table 2, which shows survival in hours of the exposed and the unexposed mosquitoes, and the mean survival rate of

TABLE 2

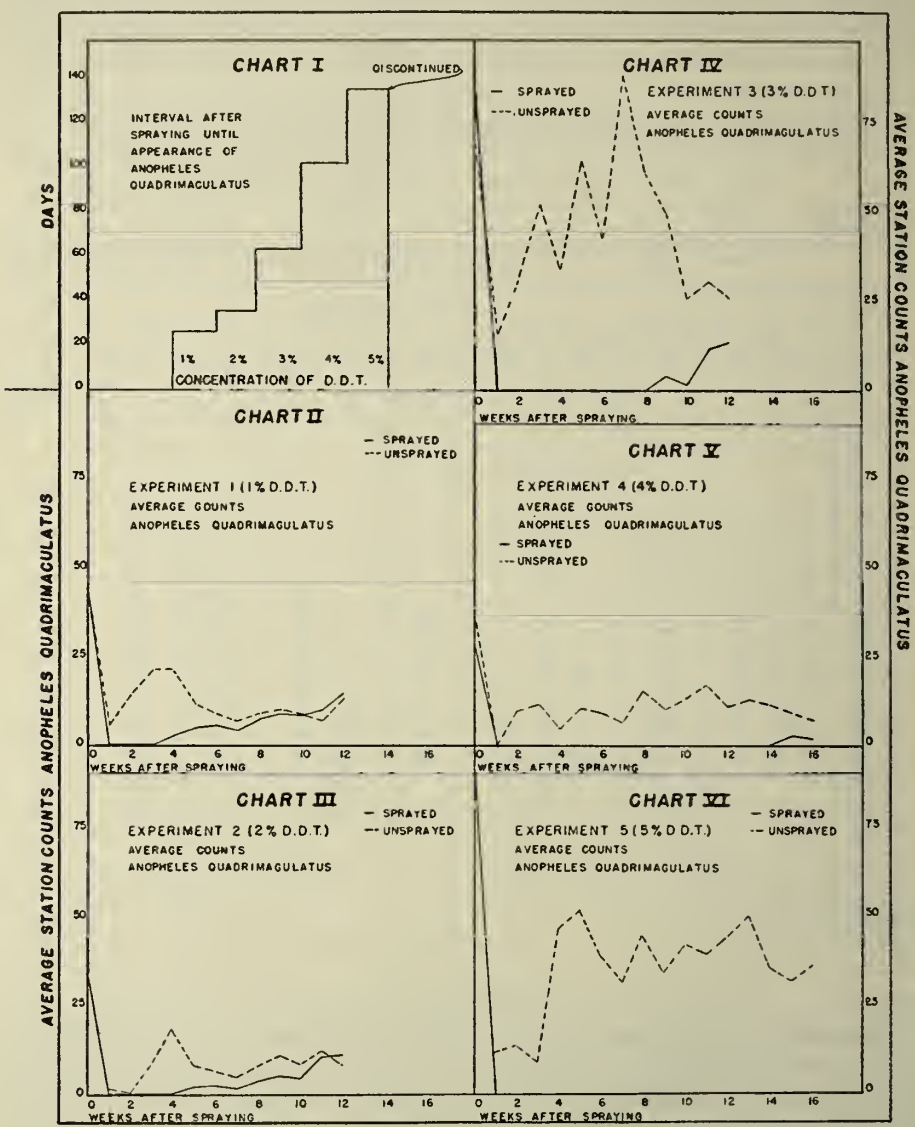
Cage tests of An. quadrimaculatus *on surfaces sprayed with varying concentrations of DDT*

HOURS AFTER EXPOSURE	1% DDT SPRAYED 73 DAYS, 3 CAGES		2% DDT SPRAYED 73 DAYS, 3 CAGES		3% DDT SPRAYED 67 DAYS, 3 CAGES		4% DDT SPRAYED 69 DAYS, 3 CAGES		5% DDT SPRAYED 66 DAYS, 3 CAGES	
	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed
	Number of survivors									
0	15	15	15	15	15	15	15	15	15	15
0.5									8	
1.0							11		2	
1.5									0	
2.0							6			
3.0							4			
4.0							0			
6.0	15	15						14		14
8.0	15	15						14		
10.0	15	15						14		
12	15	15			12			13		13
16	15	15			11			13		
20	13	15	14		9			13		
24	10	15	11		5	14		11		9
36	7	12	6	13	1	10		9		8
48	0	9	0	10	0	8		7		
60		7		7		6		5		7
72		4		5		0		2		5
84		0		1				0		0
96				0						
Mean life in hours.....	27.7	49.6	27.5	52.8	19.1	42.9	1.4	38.4	0.33	51.5

each group. It is apparent that the mosquitoes exposed to surfaces sprayed with 1 per cent and 2 per cent emulsions survived for a mean length of time exceeding 24 hours. In both cases, however, the exposed mosquitoes died at a slightly more rapid rate than did the unexposed ones, indicating some slight persistance of toxicity 73 after spraying. It is not considered, however, that the rate of kill in these two tests is satisfactory.

A comparable experiment in which mosquitoes were exposed to the surfaces sprayed with 3 per cent DDT showed a mean survival rate of 19.1 hours after exposure, which indicates a rate of kill somewhat more rapid than that in experiments with 1 per cent and 2 per cent. However, the control mosquitoes in this experiment died at a correspondingly more rapid rate than did the controls in the two former tests.

Similar tests of the surfaces sprayed with 4 per cent and 5 per cent concentrations, 69 and 66 days after spraying, produced comparatively rapid rates of kill of exposed mosquitoes as compared with the survival rate of the control mosquitoes. Mosqui-



CHARTS I—VI

toes exposed to these concentrations of DDT survived for a mean of 1.4 and 0.3 hours respectively as compared with 38.4 and 51.5 hours respectively for the control mosquitoes. It is therefore apparent that after 69 and 66 days, concentrations of 4 per cent and 5 per cent DDT emulsion remained highly toxic to *A. quadrimaculatus*.

SUMMARY AND CONCLUSIONS

Five experiments were conducted to determine the duration of toxicity of concentrations of DDT emulsions ranging from 1 per cent to 5 per cent against *A. quadrimaculatus* when employed as a residual spray. The 1 per cent concentration persisted for twenty-seven days, maintaining the sprayed surfaces free of mosquitoes, as measured by careful inspections made twice each week. Two per cent concentration kept the surfaces mosquito-free for 35 days; 3 per cent for 62 days; 4 per cent for 101 days, and 5 per cent for 135 days, at which time observations were discontinued. Mosquito counts in adjacent shelters decreased markedly in each instance after spraying, indicating that movement from one shelter to another is considerable. After the initial decrease in *A. quadrimaculatus* prevalence and their subsequent return to the sprayed and the unsprayed surfaces, the prevalence of the species was then progressively greater as time passed, except in experiment No. 5, in which no quads were found on the sprayed surface for the duration of the observations. The period of freedom from mosquitoes also increased with the increase in concentration of DDT. It appears that the efficacy of the insecticide as measured by the period of freedom from mosquitoes in these experiments, is greatly increased as the concentration is increased, up to the limit of 5 per cent used in these experiments. Econony in labor and material would favor the choice of concentration of 4 per cent or 5 per cent emulsions.

Measurements of the extent of loss of toxicity of the several concentrations were made by exposure of caged mosquitoes to the sprayed surfaces. After time intervals of 66 to 73 days following the application of DDT, concentrations of 1 per cent to 5 per cent each exhibited some degree of toxicity to *A. quadrimaculatus*. The rate of kill of 1 per cent, 2 per cent, and 3 per cent concentrations, however, can scarcely be considered satisfactory. Concentration of 4 per cent and 5 per cent, produced rapid kills of *A. quadrimaculatus* 66 and 69 days after application as a residual spray.

It is concluded that, based on the observations reported in this paper:

(a) The duration of toxicity to *A. quadrimaculatus* of DDT emulsion applied as a residual spray resulting in a residue varying from 40 to 200 mgm. per sq. ft. is proportionate to the concentration used.

(b) Application to the surfaces in one shelter markedly reduce the numbers of *A. quadrimaculatus* in adjacent shelters.

(c) Concentrations of 4 per cent and 5 per cent (160 to 200 mgm. per sq. ft.) DDT maintained sprayed surfaces mosquito-free for 101 and 135 days respectively, whereas lesser concentrations persisted for shorter and less satisfactory periods of time.

(d) Cage tests of toxicity of sprayed surfaces indicated a demonstrable toxicity for all concentrations used, from 66 to 73 days after spraying. Concentrations of 4 per cent and 5 per cent produced a rapid kill of *A. quadrimaculatus* 66 and 69 days after spraying. Lesser concentrations, 67 and 73 days after spraying killed at a less satisfactory rate.

(e) Concentrations of 4 per cent and 5 per cent (160 to 200 mgm. per sq. ft.) DDT appear to be more economical and more efficient than lesser concentrations.

THE EFFECT OF ONE PLANT EXTRACT AND OF CERTAIN DRUGS ON THE DEVELOPMENT OF *PLASMODIUM GALLINACEUM* IN *AEDES AEGYPTI*

H. A. JOHNSON AND HARVEY AKINS

Office of Malaria Investigations, Division of Tropical Diseases, National Institute of Health, Memphis, Tenn.

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Some years ago it was thought by some observers that substances existing in clover blossoms prevented the development of plasmodia in *Anopheles* mosquitoes. Although this belief was shown to be erroneous it has since been demonstrated by Osborne (1943), and Lucas and Lewis (1944), that many plants do contain inhibitory substances against microörganisms *in vitro*. Quinine, itself of plant origin, is well known to have an inhibitory action against human malaria.

In this laboratory *Plasmodium gallinaceum* is employed experimentally and, as a large colony of *Aedes aegypti* is maintained, it seemed desirable to test the effect of plant extracts and of known antimalarial drugs on the development of *P. gallinaceum* in *Aedes aegypti*.

METHODS

The general line of procedure was as follows:

Female mosquitoes were allowed to emerge from pupae into lantern gloves (not over 50 insects were kept in a single globe). As soon as emergence was complete, cotton sponges moistened with 2 per cent sugar water solution in which the test drug was incorporated, were placed on the gauze top. The blood feeding on an infected chicken was offered 4 to 6 days following emergence. The sponges were removed 24 hours previous to the blood meal in order that insects would be hungry.

After the feeding on infected chickens, engorged female mosquitoes were carefully removed to a clean gauze-topped globe and held 14–20 days to allow development of sporozoites and their migration to the salivary glands. During this interval the cotton sponges were kept wet with the sugar water containing the plant extract or the drug being tested. At the end of this period the salivary glands were dissected out and examined for the presence of sporozoites. Mosquitoes used for controls were handled, fed and examined for infected glands in exactly the same manner as those under test except that the drugs were omitted.

All mosquitoes were kept under insectary conditions of 75°–80° F with 60–70 per cent relative humidity.

The only plant extract thus far tested was made of red clover blossoms (*Trifolium pratense L.*). Three tests were carried out using the extract in saline and in tap water. In each test 14 fresh clover blossoms were ground in a mortar with 100 cc. of saline or water as noted in the table. The mixture was filtered and the filtrate was used on the cotton sponges.

No criteria were available upon which a suitable dosage of the drugs could be figured. In a pilot experiment the purely arbitrary preparation of 5 tablets of paludrine (87 mgm. of base each) per 1000 cc. of a 2 per cent sugar solution produced sporozoite free mosquitoes when fed continuously to the insects. With this in mind the initial dosages of other drugs in 1000 cc. of 2 per cent sugar water were selected more or less arbitrarily. With quinine and atabrine the dosages were figured in terms of the salt indicated in the discussion, while dosages of pamaquin, paludrine and chloroquine were figured in terms of the base.

Subsequent tests of the drugs were made by reducing the amount of drug per liter of sugar water with the purpose of determining the minimal quantities necessary to suppress the infection in the mosquitoes.

RESULTS

The results of these tests are tabulated in table 1.

Red clover. The extract was offered by the usual wet cotton sponge method. In the first and third tests no significant effect of the extract was noted. In the second test the insects all died prior to the end of the 14-day period.

Atabrine dihydrochloride. A dosage of 1.3 grams per liter of 2 per cent sugar water was found to be toxic to the insects and it had to be removed after 10 days in order to prevent death of most of the mosquitoes. Tests were carried out using one-half, one-fourth and one-eighth this dosage. With a dosage of 0.66 grams in 2 per cent sugar water a partial inhibitory effect was suggested while none was evident with the lower dosages. The figures are expressed in terms of the salt.

Pamaquin (plasmochin) naphthoate. In the dosages used, the drug did not appear to be toxic to the insects. The results of four tests suggested a partial inhibitory effect. It is to be noted that only slight inhibitory effect was found with the highest drug dosage. With a dosage of 11.2 mgm. per liter no inhibitory effect was noted. Dosage figures given are in terms of the base.

Quinine sulphate. Tests using 1.33 grams and 0.67 grams per liter apparently prevented completely the development of sporozoites. With a dosage of 0.32 grams per liter 2 out of 10 mosquitoes had positive glands compared with 11 out of 15 controls. Dosages is figured in terms of the salt.

Chloroquine base. The drug in the dosages used was very toxic to mosquitoes, only 5 insects surviving sufficiently long with the 1 gram dosage to permit examination for infected glands. Even with this toxic dosage, complete suppression of sporozoite development did not occur as one out of the five mosquitoes showed sporozoites, while 14 out of 21 controls were positive. With the 0.5 gram dosage mosquitoes did not survive sufficiently long to develop sporozoites. With a dosage of 0.25 gram per liter 7 out of 10 were positive; 0.25 grams per liter of water was apparently the largest dose that would permit survival of the insects.

Paludrine hydrochloride. The first five tests gave a complete suppression of sporozoite development as compared to 70 to 100 per cent positives in the control groups. With the two smallest dosages, 0.108 and 0.065 gram per liter, one mosquito gland out of 10 was positive in each case. The dosage is figured in terms of base.

Paludrine was not toxic to mosquitoes in any of the dosages used and seemed to

TABLE 1

Effect of red clover extract and five antimalaria drugs on development of P. gallinaceum *in* Aedes aegypti *mosquitoes*

DRUG USED	DOSAGE*	DAYS ELAPSED TIME FEEDING TO DISSECTION	NO. MOSQUITOES EXAMINED			
			Treated		Controls	
			Total	Pos.	Total	Pos.
Red clover	[1]	14	40	24		
	[1]	All Died				
	[2]	14	10	8	5	5
Atabrine dihydrochloride	1.3 g.[3]	14	10	9	10	10
	0.66 g.	16	15	3	21	14
	0.325 g.	17	10	6	15	11
	0.15 g.	18	10	10	15	12
Pamaquine napthoate	67 mg.	14	10	7	10	10
	33 mg.	16	22	5	21	7
	33 mg.	17	10	5	5	5
	22.5 mg.	17	10	5	11	11
	11.2 mg.	18	10	10	15	12
Quinine sulphate	1.33 g.	14	10	0	10	10
	0.67 g.	16	11	0	21	14
	0.32 g.	17	10	2	15	11
	0.16 g.	18	10	9	15	12
Chloroquine base	2.0 g.	All died				
	1.0 g.	16	5	1	21	14
	0.5 g.	All died				
	0.25 g.		10	7	15	12
Paludrine hydrochloride	0.435 g.	14	37	0		
	0.435 g.	19	22	0	11	10
	0.435 g.	14	10	0	10	10
	0.218 g.	16	22	0	21	14
	0.218 g.	17	10	0	5	5
	0.108 g.	17	10	1	15	11
	0.065 g.	18	10	1	15	12
	0.218 g.	26[4]	5	4	10	7

* Dosages which are expressed in grams or milligrams refer to quantity of drug dissolved in a liter of 2 per cent sugar water.

[1] Filtrate from 14 blossoms in 100 cc. normal saline (with 2 per cent sugar).

[2] Filtrate from 14 blossoms in 100 cc. tap water (with 2 per cent sugar).

[3] Atabrine removed at 10 days.

[4] This test was made with mosquitoes 21 days after infection feeding and which subsequently had been fed sugar water only. At 21 days paludrine was fed and continued for 5 days. The insects were then examined for sporozoites.

exert a very marked inhibitory action in all tests carried out when kept continuously before the insects during the period of extrinsic incubation.

A group of mosquitoes, 21 days after feeding on infected chickens, was sampled and 7 out of 10 found to have sporozoites. The mosquitoes were then fed paludrine in a dosage of 0.218 gram per liter of sugar water for a period of 5 days. Examination of the group at the end of this period showed 4 out of a total of 5 to be positive. Apparently under these conditions paludrine exhibited no lethal effect on the sporozoites.

A death rate of approximately 25 per cent occurred among the "control" insects and a somewhat higher rate among the test insects. Where the relative death rates were widely disproportionate mention has been made of the fact in the tables.

DISCUSSION

Terzian (1947) recently reported the action of various drugs on the development of *P. gallinaceum* in *Aedes aegypti*. He fed the drugs to the insects substantially as reported herein, but judged his results by microscopic examination of mosquito suspensions and by whether or not parasitemia in chickens followed the injection of ground mosquito suspensions.

The drugs used by Terzian included quinine hydrochloride, atabrine hydrochloride, plasmochin citrate and sodium sulfadiazene. He noted almost complete suppression of sporozoites with sulfadiazene as contrasted with the other drugs and those rare sporozoites found following the use of this drug were incapable of producing an infection in chickens.

Although Terzian found no inhibitory effects with quinine and atabrine, it is noted that he employed different salts of the drugs and used smaller total dosages than reported in this paper.

SUMMARY

This report presents preliminary studies on the action of red clover extract and five known antimalarial drugs on the development of *P. gallinaceum* in *Aedes aegypti*. In dosages not lethal to the mosquito, paludrine hydrochloride and quinine sulphate prevented completely the development of *P. gallinaceum* when fed continuously to the insects. Under similar conditions results following the administration of atabrine dihydrochloride and pamaquin naphthoate suggested a partial inhibitory effect. No inhibition was noted with red clover extract or with chloroquine base.

When paludrine hydrochloride was offered *Aedes aegypti* already having sporozoites no diminution in the infection rate was observed.

The authors desire to acknowledge their indebtedness to Mrs. Betty Vander Boom, Miss Mary Coode and Miss Nell Coleman for their assistance in carrying out these experiments.

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MEDICAL RESEARCH IN MALARIA: 1947

REPORT OF THE COMMITTEE ON MEDICAL RESEARCH[1]

(Received for publication 2 December 1947)

Since presentation of the report of this Committee for 1945–1946 (Report of the Committee on Medical Research, 1947), events of interest in the field of medical research in malaria include:

1. Award of the prize of the American Association for the Advancement of Science to Quentin M. Geiman and Ralph W. McKee for cultivation of malaria parasites in vitro (Geiman et alii, 1946: Science, 1947).

2. Publication of details of the extensive war-time antimalarial drug testing program (Wiselogle, 1946).

3. Announcement of pentaquine, a new antimalarial drug related to pamaquine (Lock, 1946).

Further data on paludrine (Fairley, 1947; Afridi, 1947) support the earlier views as to the great value of this drug, particularly under field conditions, but up to the present time, no single drug has as yet been developed which in addition to being effective in the treatment of clinical attacks and in suppression, is also a truly prophylactic and curative agent in all species of human malaria. The report by Fairley (1947) is of particular interest as an example of experimental work in human malaria, and for the indirect evidence brought forward to indicate the probable activity of non-erythrocytic stages of the plasmodia of man.

Recent reports on exo-erythrocytic forms have extended the knowledge of these stages in various avian malarias, but no unequivocal demonstration of their hypothetical mammalian counterparts has been put forward. Perhaps for the first time, an American worker has described several forms seen in a bone marrow culture from a patient in the prepatent stage of *vivax* infection, which bear some morphological resemblance to certain exoerythrocytic stages of avian malarias (Dubin, 1947).

An interesting contribution to the little-known field of gametogenesis in human plasmodia has been made by MacDougall (1947).

The Committee has again sent questionnaires to laboratories engaged in malaria research in the United States and abroad, and a summary of current activities in this field derived from analysis of the replies received is presented.

REPORTS FROM LABORATORIES IN THE UNITED STATES

Reports have been received from 31 laboratories engaged in medical research on malaria in the United States during 1947; another 9 laboratories which submitted reports last year did not supply data to the Committee for 1947. If these latter be

[1] Members of the Committee on Medical Research:
Harry Beckman, Marquette University.
Sterling Brackett, American Cyanamid Co.
David P. Earle, Jr. New York University.
Victor H. Haas, U. S. Public Health Service, Chairman.

included, it is evident that there are at least 40 laboratories in the United States now active in this field. Two laboratories which were included in last year's report have since ceased research in malaria.

Location. The 40 laboratories now known to be engaged in medical research are located as follows:

East and Mid-West	27
South	12
West	1

Institutions. The various types of institutions are represented as follows:

Universities	23
Government (Federal and City)	9
Pharmaceutical industries	6
Private foundations	2

Grants-in-aid. Research was supported by grants-in-aid as follows:

Government grants: to universities (including 6 laboratories not reporting to the committee in 1947)	18
Government grants: to private foundations	1
Private foundation grants: to universities	1
Private foundation grants: to government (city)	1
Industrial grants to universities	2

Federal grants-in-aid totaled approximately $250,000.

Lines of Investigation. More than one line of investigation is being followed in 14 of the 31 active laboratories reporting for 1947; the remaining 17 limit their programs to single problems. The following subjects are listed as being under active study:

Antimalarial drugs: This is the most popular field at present, as it was last year and during the war. Of the 31 laboratories currently reporting, 19 list activities in connection with synthesis or testing of antimalarial drugs; 10 indicate that their total activities are in this field; 4 of these are engaged only in preparation of drugs, testing being done elsewhere.

Immunity: Eleven laboratories are interested in various aspects of this subject. Particular studies range from serological tests to attempts at active and passive immunization.

Other investigations: Tissue culture of plasmodia is being carried out in 3 of the 31 laboratories reporting; studies on host-parasite relations are listed by 3, and on the life history (including biology) of the parasite by 3. Problems of mosquito transmission are being investigated in 3 laboratories.

Two laboratories report interest in the search for exo-erythrocytic forms in mammalian malarias; two are concerned with epidemiological problems.

Each of the following studies is a part of the program of a single laboratory: Nutritional requirements of the parasite, metabolism of the parasite, effect of blood of refractory hosts on the parasite, modification of plasmodia by environmental change, electro-kinetic charge on the parasite and the erythrocyte, cytology of plas-

modia, preservation of plasmodia by freezing, influence of nutrition on course of infection, physiological and biochemical changes produced by malaria in the warm-blooded host, overwintering of plasmodia in mosquitoes, feeding mosquitoes through membranes, and staining techniques.

Parasites and mosquitoes. The malarias and mosquitoes in use in the 31 laboratories reporting are shown in table 1.

REPORTS FROM ABROAD

Replies to questionnaires sent to workers and laboratories outside the United States numbered 22, representing 16 countries. The data submitted are summarized below:

Latin-American countries. (1) The Oswaldo Cruz Institute, Rio de Janeiro, Brazil, reports studies on evolution of human plasmodia in man and mosquito, exo-erythrocytic forms in man, monkeys, and pigeons, and general studies on plasmodia of chickens and canaries. Parasites under study include the human species, and *P. knowlesi*,

TABLE 1

PLASMODIA	NUMBER OF LABORATORIES	MOSQUITOES	NUMBER OF LABORATORIES
vivax	5	*Anopheles quadrimaculatus*	5
falciparum	5	*Anopheles albimanus*	2
malariae	4	*Anopheles freeborni*	1
cynomolgi	6	*Anopheles punctipennis*	1
knowlesi	4		
lophurae	10	*Aedes egypti*	6
cathemerium	7	*Aedes albopictus*	1
gallinaceum	6	*Aedes atropalpus*	1
relictum	3	*Culex pipiens*	2
circumflexum	1	*Culex quinquefasciatus*	1
elongatum	1		

P. gallinaceum, and Hemoproteus. Mosquitoes employed: Anophelines, *Aedes egypti*, *Culex quinquefasciatus*.

The staff of the Institute are listed as Alcides Godoy, Henrique de Beurepaire Aragao, Julio Muniz Cesar Pinto, Lobato Paraense, and Walter Oswaldo Cruz.

(2) From Cuba, reports were received from the Comision de Malaria, the Institute Nacional de Higiene, and the Escuela Sanitaria Nacional y de Medicina Tropical. Interest centers in work of an entomological nature. Staff members: Guillermo Cuervo Barrena, Concepcion Gonzalez Richard, Dr. Clerch, and Guillermo Lage.

(3) The Laboratorio de Antipaludica, San Jose de Costa Rica, Manuel Antonio Martinez, Director, is concerned with epidemiological and entomological problems.

(4) The Laboratorio de Malariologica, Ciudad Trujillo, Dominican Republic, is engaged in field work of an epidemiological and control nature, but is not a research institution. The staff comprises Luis F. Thomen, Demetrio Ganan C., Jas. D. Caldwell, Manual A. Vivas, and Jose de Jesus Ravelo.

(5) From Mexico, the Institute de Salubridad y Enfermedades Tropicales reports the following lines of investigation:

(a) Studies on anopheline mosquitoes (taxonomy, distribution, bionomics, roles as vectors, etc.).

(b) Epidemiology of malaria in Mexico.

(c) Identification of different strains of Mexican malarias.

(d) Incidence and characteristics of plasmodia in Mexican birds.

(e) Blood changes in human malaria.

(f) Action of drugs in human malaria under field conditions, and in avian malarias.

Parasites and mosquitoes used included: *P. vivax*, *P. falciparum*, *P. gallinaceum*, *P. juxtanucleare; Anopheles albimanus*, *Aedes egypti*, *Culex quinquefasciatus*.

Staff members: Enrique Beltran, Miguel E. Bustamante, Luis Vargas.

(6) The Gorgas Memorial Laboratory, Panama, is carrying out field tests of quinine, atabrine, and chloroquine, under Dr. Herbert C. Clark and Capt. Harold Trapido.

(7) The Department of Parasitology, Institute of Hygiene, Montevideo, Uruguay, reports interest in experimental studies on the infectivity of plasmodia to Uruguayan Anophelines. Principal investigator is Rodolfo V. Talice.

(8) From Venezuela, the Division de Malariologia reports work on epidemiology and entomology, and on treatment with chloroquine and paludrine. No host-parasite problems are under study at present. The staff comprises: Arnoldo Gabaldon, A. Gomez Marcano, Pablo Cova Garcia, Arturo Luis Berti, Gerardo Gonzalez, Miguel Nieto Caricedo.

Europe. From England, the following reports have been received:

(a) The National Institute for Medical Research, London. Staff: F. Hawking, W. L. M. Perry. Interested in development of sporozoites in *Plasmodium cynomolgi;* life history of *Hemoproteus kochi;* mode of action of paludrine; growth of plasmodia in tissue culture; routine drug tests against *P. gallinaceum.* Parasites and mosquitoes: *P. cynomolgi*, *P. knowlesi*, *P. gallinaceum; Anopheles quadrimaculatus*, *Aedes egypti.*

(b) London School of Hygiene and Tropical Medicine, Dept. of Parasitology. Staff: H. E. Shortt, B. Malamos, P. C. C. Garnham. Lines of investigation: exoerythrocytic cycles in *P. gallinaceum* and *P. kochi*, and hypothetical exoerythrocytic cycle in *P. cynomolgi.* Stages in the pre-erythrocytic cycle following the bites of infected *A. egypti* have been found in the brain as early as the 48th hour; cysts occurring in the livers of monkeys are thought to be the developmental stages of *P. kochi.*

(c) Molteno Institute, University of Cambridge. New Synthetic drugs are tested for antimalarial action upon *P. relictum* in canaries. Infections are induced by sporozoites (*Culex pipiens*) and by blood inoculation to immunity and drug treatment in *P. gallinaceum* and *P. relictum.* *Aedes egypti* are under study as to feeding habits. Ann Bishop, Betty Birkett, Barbara Gilchrist.

(d) Imperial Chemical Industries, Ltd., Manchester, (Biological Dept.) F. H. S. Curd, D. G. Davey. Lines of Study: Chemotherapy of exo-erythrocytic forms in *P. gallinaceum*, *P. cathemerium*, and *P. relictum;* details of action of antimalarial drugs, from studies in vitro linked with studies of distribution of the drugs in the body and their therapeutic effects. Parasites: *P. gallinaceum*, *P. lophurae*, *P. cathemerium*, *P. knowlesi.* Mosquitoes: *Aedes egypti; Culex molestus.*

From France, Dr. M. Ploye has sent a note on the interests of La Revue du

Paludisme et de Medecine Tropicale, but no reports from Laboratories engaged in malaria research have been returned to the Committee.

Last year the Committee's report included an interesting and informative letter from Dr. W. G. Venhuis describing malaria research in the Netherlands Indies. A questionnaire addressed to Dr. Venhuis this year was answered from Amsterdam by Mrs. Venhuis, advising of the death of her husband in November, 1946.

Africa. The Institut Pasteur d'Algerie reports studies on epidemiology and prophylaxis of human malaria in general, particularly in respect to North Africa. Laboratory and field studies of antimalarial drugs are being conducted. Technical direction to the antimalarial campaign in Algiers is given. Studies are also carried out on premunition in avian malaria. Parasites employed: *P. relictum*, transmitted by *C. pipiens*. Staff: Edmond Sergent, Etienne Sergent, Louis Parrot, Antoine Catanci, Henri Filey.

From Egypt, Dr. S. M. Madwar, Director of the Insect Eradication Section, Ministry of Public Health, reports that the malaria laboratory has not yet been completed.

Asia. Dr. R. B. Watson, International Health Division, Rockefeller Foundation, reports from China that medical research in malariology is not at present going forward either there or in Japan, except for a small study on paludrine in Formosa. Dr. Watson thinks it unlikely that medical research in malariology will be initiated in China "at any time in the predictable future."

The Committee received information from India before the creation of the Dominions of India and Pakistan. Undoubtedly major revisions have since been instituted, but there is no intimation as to their nature, except that almost complete changes in leading staff memberships have occurred.

The Malaria Institute of India, Delhi, in May, 1947, was interested in the testing of antimalarial drugs, and in several other fields which do not come within the scope of this Committee. Parasites and mosquitoes maintained were the following: *P. knowlesi*, *P. cynomolgi*, *P. inui*, *P. gallinaceum*, *P. cathemerium; Anopheles subpictus*, *A. annularis*, *Armigeres obturans*, *Aedes albopictus*, *Culex fatigans*. At that time, the director was Lt. Col. M. K. Afridi, but since the creation of the new Dominions, it appears that Col. Afridi is now with the Pakistan government at Karachi, and the director of the Institute at Delhi is presently Lt. Col. Jaswant Singh.

The Central Research Institute, Kasauli, was engaged in May in "search for exoerythrocytic schizogony in mammals, and studies on the fate of sporozoites. The studies have covered a histological search for EE forms in a wide range of tissues at different stages of infection, and experiments to determine the infectivity of the blood and tissues during the incubation period following sporozoite injection." Parasites: *P. cynomolgi*. Mosquitoes: *A. annularis*, *A. subpictus*. This laboratory also reported the finding of *P. vassali* in the Malabar squirrel. At that time, Lt. Col. H. W. Mulligan was director, but it now appears that the English staff are no longer in residence.

From the Institute for Medical Research, Kuala Lumpur, Malaya, a letter indicates current work on therapeutic and suppressive action of paludrine and chloroquine, and proposed studies on morphology of Malayan plasmodia, systematic clinical evaluation of new remedies, quick methods of staining, and epidemiology. Studies in control

and entomological fields, now being carried out or planned, are not within the scope of this Committee. Within the last few years, malaria parasites have not been experimentally maintained in the laboratory. Staff: J. W. Field, J. H. Strahan, J. A. Reid, J. B. D. Edeson, plus 11 technical assistants and 20 mosquito collectors and laboratory attendants.

SUMMARY

Continuing interest in medical research in malariology in the United States is indicated by the fact that approximately 40 laboratories are engaged in this field. More than a score of lines of investigation are being followed, indicating a wide range of interest.

So far as available information indicates, research in the field of medical malariology seems to be limited elsewhere in the world, except for that in England, and for one laboratory in Brazil and one in Mexico reporting active research programs. The situation in India is not now clear, although earlier in the year, a number of important research projects were under way there; how well these will be carried through under present conditions remains to be seen.

In the United States and elsewhere, interest in antimalarial drugs is still paramount, as it was during the war. The advances in chemotherapy resulting from the intense activity in this field during the war have been significant. The point to remember now is that these studies—like other research of the developmental type carried out during the war—were based upon preceding fundamental investigations whose practical importance would have been virtually impossible to foresee. There is danger that preoccupation with developmental research, as exemplified in medical malariology by the continuing enthusiasm for chemotherapeutic studies, and in the entomological field by the extensive studies on DDT, will impede the vigorous prosecution of the essential basic investigations upon which the more practical developmental progress depends.

The Committee on Medical Research, after a two-year survey of activities in this field, believes that the National Malaria Society should call upon its members (1) to encourage fundamental studies in their own laboratories, (2) to stimulate the expansion of basic research in protozoology, entomology, immunology, pharmacology, and other sciences, with a view to providing a wider background of fundamental knowledge from which ultimately better practical methods of malaria prevention and control can be developed and (3) to encourage the training of students in these fields. Every one in science today knows that we are extremely short of trained personnel; what every one must be made to realize is that we are dangerously short of basic knowledge, too. No where is this more true than in medical malariology, and only by diligent effort as individuals and as a society can we forestall the scientific stagnation that has come upon malaria research from time to time in the past, upon the heels of some arresting discovery.

LABORATORIES IN UNITED STATES

Laboratories in the United States engaged in medical research in malariology as summarized from reports submitted to the Committee on Medical Research, 1947 are listed below. The information is given in the following order: Laboratory, personnel, parasites and mosquitoes used, and lines of investigation.

American Cyanamid Co., Stamford, Conn., Sterling Brackett, Emanuel Waletsky—*Plasmodium gallinaceum*—No mosquitoes—Occasional drug testing.

California Institute of Technology, Pasadena, California, J. B. Koepfli—None—Preparation of anti-malarial drugs.

Chicago, University of—No report received for 1947.

Christ Hospital, Cincinnati, Ohio, L. H. Schmidt, H. B. Hughes—*P. cynomolgi* in *Anopheles quadrimaculatus*—Determine resemblance of *P. cynomolgi* to *P. vivax* in response to antimalarial drugs. Search for exoerythrocytic forms of *P. cynomolgi*.

Columbia University—No report received for 1947.

Emory University, Atlanta, Georgia, W. B. Redmond—*P. relictum*, No mosquitoes—Determination electrokinetic charge on surface of parasite and erythrocytes. Possible relationship of this charge with immunity. Changes in sedimentation rate during infection. Nature of immunity and factors responsible for its development.

Harvard University School of Medicine, Boston, Mass., Q. M. Geiman, R. W. McKee—*P. knowlesi* and *P. cynomolgi*, *P. vivax*, *P. falciparum*, and *P. malariae* obtained from nearby hospitals.—No mosquitoes—In vitro cultivation of malaria parasites. Nutritional requirements of malaria parasites. Metabolism of malaria parasites. Effect of antimalarials and other parasite antagonists. Immunity studies (antibody protein)

Harvard University, Converse Laboratory, Cambridge, Mass.—*P. lophurae*, No mosquitoes—Synthesis and study of antimalarial drugs (Naphthoquinones).

Iowa State College, Ames, Iowa, E. R. Becker—*P. lophurae*, No mosquitoes—Testing of antimalarial drugs.

Johns Hopkins University—No report received.

Lederle Laboratories, Pearl River, N. Y., Redginal I. Hewitt—*P. lophurae*, *P. cathemerium*—Experimental chemotherapy.

Lilly Research Laboratories, Indiananapolis, Ind., K. K. Chen, C. L. Rose—*P. lophurae*, *P. relictum*—Testing of antimalarial drugs.

Marquette University, School of Medicine, Milwaukee, Wisconsin, Harry Beckman—*P. cathemerium*, *Culex pipiens*—Effect of blood of refractory hosts on plasmodia.

Maryland, University of—No report received for 1947.

Merck Institute for Therapeutic Research, Rahway, New Jersey, Hans Molitor, A. C. Cuckler, —*P. gallinaceum*, *P. lophurae*, *P. cathemerium*, *Aedes egypti*—Testing of anti-malarial drugs. Influence of nutrition on course of malaria.

Michigan, University of, School of Public Health—No report received for 1947.

New York City, Public Health Research Institute of Jules Freund, K. J. Thomson, *P. lophurae*, *P. cathemerium*, *P. knowlesi*—Immunization with aid of adjuvants.

New York University Research Service, Goldwater Memorial Hospital, Welfare Island, N. Y. R. W. Berliner—*P. falciparum*, No mosquitoes—Testing of antimalarial agents in vitro.

North Carolina, University of, School of Medicine, Chapel Hill, N. C., J. C. Andrews, M. M. McEwen—No parasites or mosquitoes—Relation between dietary deficiencies and the rate of quinine metabolism in white mice.

Northwestern University, Dept. of Chemistry—Evanston, Ill., Byron Riegel, R. H. Baker—No parasites or mosquitoes—Preparation of new antimalarial drugs. Testing done at Cincinnati and Washington.

Northwestern University Medical School, H. R. Jacobs—*P. lophurae*—No mosquitoes—Immunization of ducks against inoculation malaria.

Notre Dame, University of, Notre Dame, Indiana, K. N. Campbell—No parasites or mosquitoes—Synthesis of anti-malarial drugs. Testing to be done at Iowa State College.

Parke-Davis & Co.—No report received for 1947.

Rutgers University Bureau of Biological Research, New Brunswick, N. J., Leslie A. Stauber—*P. lophurae*, *P. cathemerium*—Use of parasites freed from erythrocytes as antigens in serological tests, to determine antigenic specificity of various species of avian plasmodia.

ROCKEFELLER FOUNDATION—No report received for 1947.

SQUIBB INSTITUTE, New Brunswick, N. J.,—No research in malaria at present.

STATION FOR MALARIA RESEARCH, Tallahassee, Fla., Work terminated at end of 1946.

STERLING-WINTHROP RESEARCH INSTITUTE, Rensselaer, New York, E. W. Dennis,—*P. lophurae*—Testing of anti-malarial drugs.

SYRACUSE UNIVERSITY, DEPT. OF ZOOLOGY, Syracuse, N. Y., R. D. Manwell, P. Feigelson—"Most of the avian species of plasmodia." No mosquitoes at present, but expect to have later. Canaries, ducks, chickens, and pigeons now used.—Cultivation of avian plasmodia. Modification in staining methods. Problems of transmission.

TENNESSEE, UNIVERSITY OF, COLLEGE OF MEDICINE, Memphis, Tennessee, R. R. Overman, T. S. Hill, R. L. Laird, I. N. Dubin, Y. T. Wong, A. D. Dulaney, Hudson Jost—*P. vivax*, *P. falciparum*, *P. knowlesi*, *P. cynomolgi*, *P. gallinaceum* in chicks and embryos, *Anopheles quadrimaculatus*, *Aedes egypti*—Correlated physiology and biochemistry of simian and human hosts; Measurements of blood and plasma volume; NaSCN vol.; fluid intake; renal output; Na, K, Cl determination on blood plasma, red cells, urine; EEG, EKG, polygraph recordings, liver function, antigen antibody reactions, plasma protein. Search for exoerythrocytic forms (tissue cultures and fluorescent microscopy). Cultivation of ee forms (*gallinaceum*). Studies on host hypersensitivity. Host parasite relations.

TENNESSEE VALLEY AUTHORITY, Wilson Dam, Alabama, E. Harold Hinman, A. D. Hess, G. E. Smith, Lois Seamans,—No parasites maintained at Wilson Dam. Mosquitoes (*A. quadrimaculatus*) produced. These are infected at Memphis—Over-wintering of human plasmodia in *Anopheles quadrimaculatus*. Epidemiological investigations.

TEXAS, UNIVERSITY OF—No report for 1947.

TULANE UNIVERSITY—No report for 1947.

U. S. ARMY, ARMY MEDICAL CENTER, Washington, D. C., John F. Kent (Serology), W. G. Jahnes (Parasitology),—*P. cynomolgi*, *P. knowlesi*, *P. lophurae*, *P. cathemerium*, *P. circumflexum*, *P. elongatum*, *P. relictum*, *A. quadrimaculatus*, *A. albimanus*, *A. egypti*, *A. albopictus*, *A. atropalpus*, *C. pipiens*, *C. quinquefasciatus*—Feeding of mosquitoes through fish skin membranes. Laboratory mosquitoes as vectors. Immune relationships between avian malaria species. Improvement of larval foods. Complement fixation in human malarias (sera supplied by NIH).

U. S. NAVY MEDICAL RESEARCH INSTITUTE, Bethesda, Md., L. A. Terzian—*P. gallinaceum*, *P. cynomolgi*, *A. egypti*—Studies on immunity. Life history studies.

U. S. PUBLIC HEALTH SERVICE, NATIONAL INSTITUTE HEALTH, Columbia, S. C. M. D. Young, R. W. Burgess, *P. vivax* (St. Elizabeth), *P. malariae* (USPHS), *P. falciparum* (McLendon & Santee-Cooper), *A. quadrimaculatus* (Q-1 & Q-5), *A. m. freeborni* (F-1), *A. albimanus* (A-2), *A. punctipennis* (P-5 & P-6), *A. c. crucians* (C-5 & C-2) Parasitology in man & mosquitoes. Immunity in human malaria. Host-parasite relationships. Transmission problems. Susceptibility of various species of mosquitoes to different species of malaria. Cytology of plasmodium. Malaria therapy of syphilis. Antimalarial drugs (in cooperation with other laboratories).

U. S. PUBLIC HEALTH SERVICE, NATIONAL INST. OF HEALTH, Memphis, Tennessee, V. H. Haas, T. H. Tomlinson, H. A. Johnson, F. L. Knowles, Aimee Wilcox—*P. gallinaceum*, *P. cynomolgi*, *A. egypti*, *A. quadrimaculatus*—Active and passive immunization. Clarification of developmental cycle particularly from standpoint of exo-erythrocytic development. Modification of plasmodia in refractory animals. Experimental epidemiology. Study of substances antagonistic to plasmodia in the mosquito. Organisms parasitic on the mosquito.

U. S. PUBLIC HEALTH SERVICE, NATIONAL INSTITUTE OF HEALTH, Milledgeville, Ga., D. E. Eyles—*P. vivax* (Chesson), *P. falciparum* (Santee-Cooper) *P. malariae* (Trinidad), *A. quadrimaculatus*—Other mosquitoes occasionally—Host-parasite relationship between man and mosquito and parasite. Factors affecting intensity of mosquito infection. Drug testing in cooperation with other laboratories.

U. S. PUBLIC HEALTH SERVICE, NATIONAL INST. HEALTH, Washington, D. C., (Bethesda), G. R. Coatney, W. C. Cooper, E. S. Josephson, W. B. Culwell, H. L. Trembley—*P. gallinaceum*, *A. egypti*—Development of curative and prophylactic drugs against *vivax*. Development of better

suppressive drugs. Biology of *vivax* (St. Elizabeth & Chesson). Development of curative test against *gallinaceum*, Antimalarial drug screening in chicks.

U. S. Public Health Service, Communicable Disease Center, Atlanta, Georgia, V. B. Link, S. W. Simmons, G. H. Bradley—No live parasites or insectary maintained—Laboratory diagnosis, epidemiology, control, prevention of malaria.

Virginia, University of—No reports for 1947.

Washington University, School of Medicine, St. Louis, Missouri, G. M. Saunders, Virgil Scott—*P. vivax* (Pacific strains), *P. malariae* (Tuskegee)—Preservation of human plasmodia by freezing.

Wisconsin, University of, Dept. of Pharmacology, Madison, Wisconsin, A. L. Tatum—*P. cathemerium*—No mosquitoes—Chemotherapy, Certain aspects of immunity.

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COST RECORDS IN MALARIA CONTROL

REPORT OF THE COMMITTEE ON SANITARY ENGINEERING[1]

(Received for publication 2 December 1947)

The designated duties of this Committee are to study and report on the engineering features of malaria prevention and control, and to *collect, distribute and encourage the keeping of adequate cost records on drainage and larviciding.*

The attention of the Committee for the past 2 years has been focused on the subject of cost records. In reviewing past and current records, it has been impressed by the need for standardization in terminology and units of measurement in the recording and reporting of physical accomplishments and resultant cost data for mosquito control; and by the prevailing lack of such standards. This is in marked contrast to other engineering practices and to the relatively precise systems of definition and nomenclature followed in the medical, entomological and other scientific fields of malariology and malaria control practice. Lack of standardization has impeded the accumulation of a literature of usable cost experience interchangeable between states and countries and comparable from one year to another.

Toward rectification, the Committee has been actively engaged for 2 years in the preparation of a proposed code for the recording and reporting of such data. In addition to meetings and correspondence within the Committee membership, the engineering membership of the Society has been circularized extensively, and the technical advice and endorsement of other societies and organizations obtained. It now submits by attachment a final draft of this Code for appropriate action by the Society, proposing that it be termed: Code recommended by the National Malaria Society for the Reporting and Estimating of Costs and Physical Accomplishments in Mosquito Control.

We offer the following in further comment and explanation:

1. The Committee does not anticipate that any system of relatively precise recording will, or even should, be adopted by all mosquito control organizations for use in all situations. The inherent character of many very small scale local programs precludes this possibility. Rather, the code has been prepared with these usages in mind:

   a. *Primary*—For general use in the professional literature, including the periodic and special reports of the more highly integrated and specialized mosquito control organizations.

   b. *Secondary*—For operational use by any mosquito control organizations, including state and federal health services, which may wish to adopt uniform reporting and recording practices. (Such adoption in some instances may well be selective rather than jurisdiction-wide, i.e., confined to local projects under resident engineering management, or having special demonstration significance, etc.)

2. Preparation of a *reporting form* listing all items of performance and expenditure

[1] Members of the Committee on Sanitary Engineering: C. C. Kiker, David B. Lee, R. E. Dorer, H. W. Von Hovenberg and John M. Henderson, Chairman.

has been considered and discarded as infeasible. This decision has dictated adoption of a code of reporting terms and units for insertion in a simple reporting form or comparable tabular classification.

3. It is intended that potential usage of the code extend beyond the continental United States. In content, it includes mosquito performance items which are important in other areas but not in this country. Metric units may be substituted for English units, and translation into other languages is contemplated.

4. Emphasis is placed on the recording and reporting of expenditures in physical, rather than monetary, terms. Both categories are recognized: monetary values for summarization, to permit the resolution of all expenditures into a single figure; and to record those costs which otherwise cannot be classified conveniently. Physical expenditures however, are essential for analysis and the consequent utilization of acquired experience. Hourly wage rates for unskilled labor have varied from 5¢ to 75¢ in a single locality in this country within a 15-year period, a 15-fold range. Currently, unskilled hourly wage rates in different countries range from 6¢ in Haiti and some of the British West Indies, to 70¢ to $1.50 in this country. Under these circumstances, a reported cost of 30¢ per cubic yard for earth excavation by hand labor at another time or place has little practical significance in malaria control practice, but an experience of ½ cubic yard per man-hour has. The variable relationship between physical and monetary expenditures is not confined to personal services, but, as is well known, includes materials, supplies and equipment.

The Committee considers that the standardization and recognition of physical expenditures as the basic standard of cost reporting and cost comparison is the most vital single principle involved in the code.

5. While the purpose of the code is to encourage conformance with a standard system of classification, the presentation or publication of valuable data on different reference planes would not be precluded per se by code adoption. The code would have comparison value even where not followed. When the sub-itemization indicated cannot be furnished, the quality of the data and recording procedures is revealed. Where the data are of a quality equal to code standards, a reference base for explanation is provided by the code.

6. In the interest of accumulating a larger sum of experience data and to encourage a more comprehensive utilization, the code has not been limited to anti-anopheline activities. In the interest of disease prevention, data on the cost of controlling other mosquito vectors of disease is desired and needed. Costs and methodologies employed in controlling non-disease transmitting mosquitoes have an applicability in the control of mosquito-borne diseases, including malaria, and vice versa. To encourage use of the code by organizations having a larger scope of interest than anopheline control, certain non-anopheline reporting terms should be included.

7. Units of measurement have been selected which reflect prevailing job practices for the type and scale of operations most frequently encountered. (Examples: the unit for hand labor clearing is "thousand square feet", while the unit for mechanical clearing is the "acre". The linear and square yard have been generally avoided, since they require the conversion of measurements made in feet. Conversely, the

cubic yard is accepted, because it is practically the exclusive unit used in this country for the mensuration of earth excavation and fill.)

8. While designed principally from the standpoint of physical performance items and related expenditures incurred in project execution, the classification may also be utilized for the advanced estimation of costs and for the inclusion of developmental and intangible expenditures.

Reference Code for Use in the Recording, Estimating and Reporting of Physical Accomplishments and Costs in Mosquito Control Operations[2]

Note: The purpose of this Code is to furnish standardized classifications of terms which can be used in whole or in part on any suitable reporting or estimating form or table. If the Code is translated into other languages, metric units of physical measurement shall be substituted for English Units.

PART ONE—CLASSIFICATION OF PHYSICAL ACCOMPLISHMENT ITEMS AND THEIR UNITS

I. Drainage Construction—(Depth of excavation, 1 ft. or greater)
- A. Hand excavation—cu. yd.
  - 1. Earth Excavation—dry—clay 1–4 ft. cut
  - 2. Earth Excavation—dry—clay 4–6 ft. cut
  - 3. Earth Excavation—dry—clay 6–8 ft. cut
  - 4. Earth Excavation—dry—clay 8–10 ft. cut
  - 5. Earth Excavation—dry—clay over 10
  - 6–10. Same as 1–5, but Sand & Sand Clay
  - 11–15. Same as 1–5, but Wet
  - 16–20. Same as 6–10, but Wet
- B. Machine Excavation—Dragline—cu. yd.
  - 1–10. Wet and Dry Excavation separated, but soils not classified.
- C. Machine Excavation—Other—Specify—cu. yd.
  - 1–10. (Same as for Dragline)
- D. Excavation by Explosives—cu. yd. (Mention ditch cross-section, lbs. of explosive used per cu. yd., strength and type of explosive, planting pattern and whether explosions are propagated.)
  - 1. Earth—Cleared and stumped Right-of-Way
  - 2. Earth—Timbered Right-of-Way
  - 3. Earth—Channel Improvement
  - 4. Rock—Specify Type
- E. Clearing—hand—(includes disposal)—M sq. ft.
  - 1. Small timber—4″ diam. & under
  - 2. Other Timber—including mixed sizes, some under 4″
- F. Clearing—Mechanical (by power saws, tractors, power rakes, etc.)—Acre
  - 1. Flat slopes—10% & under
  - 2. Steep slopes—over 10%

[2] Attachment to the report of the Committee on Sanitary Engineering.

G. Grubbing—Hand—M sq. ft.
   1. Dry—roots only
   2. Wet—roots only
   3. Dry—including stumps
   4. Wet—including stumps
   5. Special—(For large stumps, unevenly distributed, report separately, using "stump" as a unit.)

H. Stumping—Dynamite or Mechanical (Specify)—Acre (See Grubbing for Hand Stumping)
   1. Light and Medium
   2. Heavy
   3. Special (See IG5)

I. Culvert Installation—Pipe
   1. Open Cut—Unshored (Report excavation in appropriate section). Report by type of pipe, Lin. Ft. & by pipe diameter in inches.
   2. Open Cut—Shored (Preceding remarks apply)
   3. Jacking (No excavation). Report by Lin. Ft. & by diameter in inches.
   4. Tunnelling or mucking (w. or w/o jacking) (Include excavation). Report by Lin. Ft. & by pipe diameter in inches

J. Culvert Installation—Box
   1. Monolithic Concrete—cu. yds. concrete (Specify dimensions. Exclude Excavation)
   2. Wood—F. B. M.
   3. Masonry (Specify type)—Cu. yd.

K. Sub-drainage—Lin. Ft. by diameter of pipe or passageway in inches
   1. Pipe—Machine Excavation
   2. Pipe—Hand Excavation
   3. French Drain—Stone or Gravel
   4. French Drain—Other

L. Ditch Lining—(Mention thickness & Type)—sq. ft. lined surface
   1. Monolithic Concrete
   2. Pre-cast concrete or hard burned clay, production
   3. Pre-cast concrete, transportation & installation
   4. Masonry (Brick or Stone)
   5. Bituminous

M. Sodding—M sq. ft.
   1. Blanket
   2. Strip
   3. Sprigged
   4. Seeded

N. Grading—Hand—M sq. ft. (Av. excavation or fill under 1 ft.)
   1. Ditch Section—General
   2. Ditch Section—Sub-grading for Lining
   3. Land Surface

O. Grading—Machine—Acre (Av. excavation or fill under 1 ft.)

1. Drag Pan
2. Bulldozer
3. Road Scraper
4. Hand (M sq. ft.)

P. Filling—cu. yd.—Av. depth 1 ft. or more. (Mention average haul)
1. Truck (2 cu. yd. & under)
2. Heavy Truck & Load-lugger (over 2 cu. yd.)
3. Carry-all
4. Bulldozer
5. Hydraulic
6. Hand, w. or w/o wheelbarrow

Q. Levees. Cu. yd. (Specify section)
1. Fill, hand or machine (specify). Report for compacted section.
2. Rip-rap. Sq. yds. (Mention size & type of facing and wgt. per sq. yd. surface covered.)

R. Special Structures (Pumphouses, catch basins, headwalls and wing walls, piling, tide gates, jetties, automatic siphons, retaining walls, ditch checks, aprons, grade monuments, etc.) Report by nearest terms and units in general construction practice.

II. Recurrent Activities

A. Vegetation Removal—Acre (Mention Predominant Plant Species)
1. Plant Water Surface—Mechanical—acre
2. Plant Water Surface—Chemical—acre
3. Plant Water Surface—Hand—acre
4. Plant Land Surface—Mechanical—acre
5. Plant Land Surface—Chemical—acre
6. Plant Land Surface—Hand—acre
7. Plant Land Surface—Burning—acre
8. Coppice—Chemical—acre
9. Coppice—Hand—acre

B. Ditch Cleaning & Related Clearing—M. sq. ft.
1. Ditch Section
2. Banks & Access Paths

C. Larviciding—(Mention Dosage Rate)
1. Broad Surface (Acreage)—acre
   a. Plain Oil—Hand
   b. Plain Oil—Power—Specify
   c. DDT Oil Spray—Hand
   d. DDT Oil Spray—Power—Terrestrial
   e. DDT Oil Spray—Power—Airplane
   f. DDT Oil Aerosol—Hand
   g. DDT Oil Aerosol—Power-Terrestrial
   h. DDT Oil Aerosol—Power—Airplane
   i. Other Oil—Hand (except DDT) (Describe)
   j. Other Oil—Power (except DDT) (Describe)

k. DDT Dust—Hand
l. DDT Dust—Power—Terrestrial
m. DDT Dust—Power—Airplane
n. Paris green—Hand
o. Paris green—Power—Terrestrial
p. Paris green—Power—Airplane
q. Other Dusts (Describe)

2. Linear Application (Ditches, Narrow Shorelines, etc.)
   a. Plain Oil—Hand
   b. Plain Oil—Power—Specify
   c. Other Oil—Hand (except DDT) (Describe)
   d. Other Oil—Power (except DDT) (Describe)
   e. DDT Oil Spray—Hand
   f. DDT Oil Spray—Power—Terrestrial
   g. DDT Oil Spray—Power—Airplane
   h. DDT Oil Aerosol—Hand
   i. DDT Oil Aerosol—Power—Terrestrial
   j. DDT Oil Aerosol—Power—Airplane
   k. DDT Dust—Hand
   l. DDT Dust—Power—Terrestrial
   m. DDT Dust—Power—Airplane
   n. Paris green—Hand
   o. Paris green—Power—Terrestrial
   p. Paris green—Power—Airplane
   q. Other Dusts (Describe)

Note: For other synthetic organic larvicides competitive to DDT, substitute name and report under DDT classifications above.

D. Adulticiding (Mention Dosage Rate & Formula)
   1. Building Applications—(M sq. ft.)
      a. Residual Spray (specify)—Hand—(Wall Applied Sprays)
      b. Residual Spray (specify)—Power—(Wall Applied Sprays)
      c. Contact Spray (specify)—Hand (Any Interior Space Spray)
      d. Contact Spray (specify)—Power—(Any Interior Space Spray)
   2. Space Application—Exterior—All types
      a. Hand—M sq. ft.
      b. Power—Terrestrial—Acre
      c. Airplane—Acre

E. Maintenance—Non-anopheline (Use previous listings where applicable)
   1. Catch Basins Treated—Give No. & Mention Larvicide & Type of Conveyance
   2. Premise Inspections—No.
      a. Breeding
      b. Potential
      c. No Breeding
      d. Total

e. Ration a/d

3. Premise control

a. No. Premises Treated

b. No. Primary Breeding Places Treated

c. No. Primary Breeding Places Eliminated

Note: This classification is not intended to serve as a basis for an intra-project reporting form for intensive Aedes aegypti control work.

III. Mosquito Proofing—No. Bldgs. (Mention av. size)

A. Screening Doors & Windows only (Mention av. no. each per building)

B. Complete mosquito-proofing (Mention type and condition of bldgs. and types of mosquito-proofing installed.)

General Note: All personal services shall be reported both in man-hours and in monetary cost or value. All equipment services shall be reported in machine hours or team hours and monetary cost or value. Items reportable only in monetary value shall be exclusive of the above. They include non-equipment rentals, incorporated equipment and materials, land purchase, expendable supplies, etc. Itemized breakdown of monetary cost or value of personal services and equipment services is not indicated.

A. Personal Services—man-hours. Where practicable, sub-divide personal services into the categories of Idle Time and Active Time. Idle Time shall include Paid Time during Absence from work of any type, including time lost due to weather and delays. Active Time for Items T-1 to T-5 inclusive should be sub-divided into Productive Time and Overhead Time, the term "Overhead Time" to include travel on foot or in vehicles and other time factors which are an essential part of the job operation but are not directly productive in physical accomplishment or related supervision.

1. Labor—Unskilled and Semi-skilled
2. Labor—Skilled
3. Labor—Overhead Services (Watchmen, Clerks, Timekeepers)
4. Inspection—Job Inspectors, Entomological Inspectors, etc.)
5. Supervision—Job Site (Foremen, Superintendents, and others)
6. Engineering—Non-supervisory (Survey parties, etc.)
7. Biological Services—Professional
8. Supervision—General—Offsite
9. Incidental Overhead Services—Educators, Census Takers, etc.

B. Equipment Services (All items to be reported in machine hours except where otherwise noted).

1. Air Compressors—specify size
2. Airplanes—Ferrying & Applying Times—State carrying capacity & cruising speed
3. Carry-alls—Specify capacity in cu. yds.
4. Concrete Mixers—Specify size
5. Draglines & Cranes—Light—$\frac{3}{8}$ & $\frac{1}{2}$ cu. yd.
6. Draglines & Cranes—Medium—$\frac{5}{8}$ & $\frac{3}{4}$ cu. yd.
7. Draglines & Cranes—Heavy—1 yd. & over

8. Dredges—Dipper
9. Dredges—Hydraulic—8″ and under
10. Dredges—Hydraulic—over 8″
11. Pile Drivers—Jack Hammer or Air Hammer
12. Portable Pumps—Power—2½″ and under
13. Portable Pumps—Power—3″ to 5″
14. Portable Pumps—Power—6″ and over
15. Shovels and Back-Hoes—Power
16. Sprayers & Dusters—Power—State size
17. Teams—team hours
18. Tractors—Wheeled
19. Tractors—Track type—light (D-4 & under)
20. Tractors—Track type—medium (D-5 & D-6)
21. Tractors—Track type—heavy (above D-6)

Note: Report bulldozers and angle-dozers under tractors

22. Trenching Machines—Wheel or Ladder Type
23. Vehicles—Passenger Cars & Station Wagons—miles
24. Vehicles—Light Trucks—¼—¾ ton
25. Vehicles—Medium Trucks—1 to 1½ ton
26. Vehicles—Heavy Trucks—2—4 tons
27. Vehicles—Very Heavy Trucks—5—25 tons

C. Supplies and Materials. The following are reporting units and classifications to be used for some of the more commonly used supplies and materials. Quantity, unit price and total price should be reported for all items. Additional reporting items are listed in some instances opposite the Accomplishment Item in Part One.

1. Pipe—State type and diameter
2. Portland Cement—Sack
3. Aggregate—Fine—ton
4. Aggregate—Coarse—ton
5. Aggregate—Bank run—Mixed—cu. yd.
6. Piling—Round—Report diam. & unit length
7. Piling—Sheet—State weight & unit length
8. Rip-rap—State size and tonnage
9. Lime—hydrated—ton
10. Paris green—lb.
11. Oil—gallon
12. DDT & other insecticidal powders—lb.
13. Solvents & emulsifiers—gallon
14. Lumber—M B M
15. Dynamite—lbs. State strength & type
16. Caps, blasting—State whether plain or electric. For electric, mention lead wire length

D. Miscellaneous Items. Item A provides a complete classification of all personal services involved in the pre-construction, construction, and post-construction stages of a project. Classifications in Items B and C include only those equipment, supply

and material items which are most commonly encountered in project operations, since it is obviously impracticable to include all. This list may be expanded in each project office to meet local conditions.

The following objective classification of account headings will serve as a guide in recording miscellaneous items of expenditure:

1. Travel
2. Freight and Hauling
3. Communication Service
4. Rents and Utility Service
5. Printing, Binding and Records
6. Contractual Services not classifiable under Items A, B and C
7. Land
8. Reserve for Depreciation or Maintenance with Time Limit
9. Workmen's Compensation and Related Expenses

Items of expenditure incurred other than in the operating period of the project, or not chargeable to specific items of physical accomplishment, may be charged under the following headings, as applicable:

1. Preliminary Survey
2. Preparation of Plans, Specifications, Estimates and Work Schedules
3. General Overhead—Construction Stage
4. Project Liquidation

MEDAL OF MERIT AWARDED E. F. KNIPLING

"For exceptionally meritorious conduct in the performing of outstanding services to the United States," Dr. Edward F. Knipling, as Director of the Orlando station of the Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture during the war period, was awarded the Medal of Merit May 20, 1948, by the Department of National Defense. The citation, signed by President Harry S. Truman, which accompanied the award stated that Dr. Knipling "planned and directed investigations for the development of insecticides and repellents for the prevention of insect-borne diseases in the United States Army. As a result of these studies the Armed Forces adopted the use of DDT for the control of insect-borne diseases and new uses and equipment were developed for the dispersing of insecticides by new methods, including the airplane spraying of infested areas. All these research contributions were of inestimable value to the health and morale of United States troops, as well as to the Nation as a whole."

The Medal of Merit, awarded only to civilians, and the Legion of Merit, awarded to military personnel, were authorized by Public Law 671, 77th Congress, approved July 20, 1942, for the purpose of rewarding outstanding and meritorious conduct in the performance of duties in furtherance of the war efforts of the United Nations. The award stems from the Badge for Military Merit, America's oldest decoration, established by George Washington in 1782.

Dr. Knipling was notified early this year that he was to be awarded Great Britain's highest decoration to civilians, the King's Medal for Service, for the part he carried on in relation to the same investigations.

CORRECTION

On page 163 of the June 1948 issue, preceding the subheading *General Note* and following the 11th line from the top of the page, should be inserted the heading:

PART TWO—CLASSIFICATION AND DEFINITIONS OF EXPENDITURE ITEMS